THE EFFECT ON CROSSING OVER AND NON-DISJUNCTION OF X-RAYING THE ANTERIOR AND POSTERIOR HALVES OF DROSOPHILA PUPAE

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THE PROBLEM

Investigations on the effects of X-rays on Drosophila which have been conducted by the writer and his co-workers at UNION COLLEGE (MAVOR 1921, 1922, 1923, 1923a, 1924; MAVOR and SVENSON 1924) have shown that X-rays induce non-disjunction and modify the crossover value in certain regions of the first and second chromosomes. At that time the writer remarked that "there is therefore in this effect of X-rays on the germ-cells a very clear case of an external agent which modifies the mechanism of inheritance in such a way that a permanent effect is produced on the germ-cells." The work has further shown that the effect of the X-rays is probably not directly on the process of crossing over. It remains to prove, if possible, whether the induction of non-disjunction and the modification of the crossover value is due to a direct effect of the X-rays on the germ-cells or to a general effect of the X-rays on the physiological condition of the fly.

When a beam of X-rays encounters matter it becomes scattered so that bodies not in the direct path of the rays receive a significant amount of radiant energy; it is, therefore, not possible to confine the treatment to either a single organ or a small portion of the animal. This difficulty is greater in the case of a small organism, such as the fruit fly, than in a larger animal. In the experiments to be described the attempt has been

made to compare the effects of irradiation of the anterior and posterior halves of the female in the late pupal stages. Since the ovaries are confined to the posterior part of the abdomen this amounts to irradiation in one case of a half of the animal containing the reproductive organs and in the other case of a half not containing these organs.

TECHNIQUE

The technique of the X-ray treatment and culture of the flies was the same as that used in previous experiments and described in reports of these (MAVOR 1924). It is necessary to dwell only upon the method by which one half of the pupa was exposed.

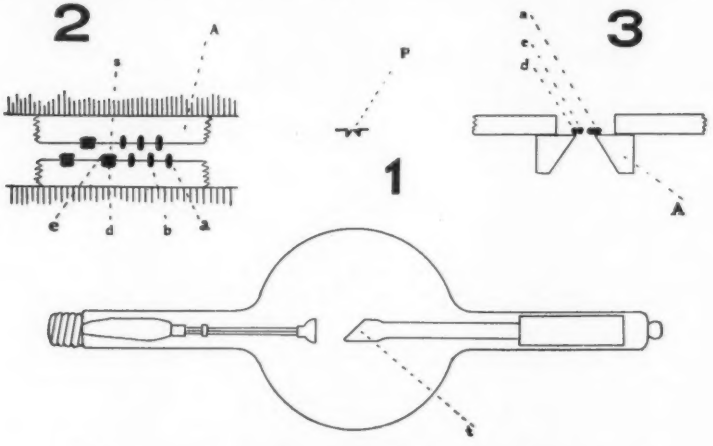

FIGURE 1.—General view of apparatus used in exposure of pupae to X-rays. *t*, target of Coolidge X-ray tube, *p*, pupae to be exposed.

FIGURE 2.—View looking down on silver bars used to shield parts of pupae from exposure. *A*, silver bar, *s*, slit between the two bars, *a*, pupa with anterior half exposed, *b*, pupa with posterior half exposed, *d*, shielded pupa, *e*, pupa entirely exposed.

FIGURE 3.—Cross section of silver bars and lead box. *A*, silver bar, *a*, pupa in position to have anterior half X-rayed, *d*, shielded pupa, *e*, pupa in position to have entire body X-rayed.

The arrangement of the apparatus for the treatment of the pupae is shown in figures 1–3. Two silver bars, *A*, figures 2 and 3, 100 mm long by 6.5×6.5 mm in cross section, with one edge beveled were held parallel to each other at a distance of 3.2 mm, with their beveled edges downward. These silver bars were held vertically over a Coolidge tube, the target, *t*, figure 1, of which was directed directly upward. On the top of the silver bars was placed a piece of moist "onion skin" paper. The pupae, *p*,

figure 1, were arranged on this paper under the binocular microscope, light being transmitted through the slit between the bars and enabling the adjustment of the pupae so that either the anterior half, *a*, figures 2 and 3, or the posterior half, *b*, figure 2, or the entire pupa, *e*, figures 2 and 3, was exposed to the direct radiation. When the entire pupa was exposed it was held with its axis parallel to the silver bars and with one side exactly over the edge of the bars. Touching this pupa and on top of the silver bar was laid another pupa, *d*, figures 2 and 3, also parallel to the bar. The latter pupa, which is called "shielded," received no direct radiation but was exposed to approximately the same secondary radiation as the posterior half of the pupae in which the anterior half was exposed to the direct radiation. The shielded pupae therefore serve as indicators of the effects of secondary radiation. In experiments 320, 322 and 330, a number 1 coverglass was placed under the onion skin paper and above the silver bars; the thinness of this, approximately .1 mm, introduced scarcely any difference in the X-ray dosage, and it enabled a more accurate placing of the pupae over the silver bars. There are, therefore, to be considered pupae submitted to the following different treatments:

a—anterior X-rayed
b—posterior X-rayed
c—control, not in the X-ray chamber
d—shielded
e—entirely X-rayed

The data are derived from four experiments, numbered 315, 316, 320, and 322. The technique was precisely the same in each of the experiments but the flies used in them had a slightly different history.

CROSSING OVER

The allelomorphs used in these experiments include white (*w*) and eosin (w^e) eye color placed on the latest map by MORGAN, BRIDGES, and STURTEVANT (1925) at 1.5, and miniature (*m*) and long (+) placed at 36.1. This gives 34.6 as the normal crossover value between the two regions. In these experiments no attempt has been made to determine or adjust for double crossing over. The crossover values determined from our counts are therefore relatively low. In our previous experiments (MAVOR 1923) involving crossing over between these two loci the total of all our counts of controls (12,561 flies) gives a mean value of 28.65.

PLOUGH (1917) has shown that neither temperature, moisture, amount of food, nor a number of chemical agents cause any appreciable change in

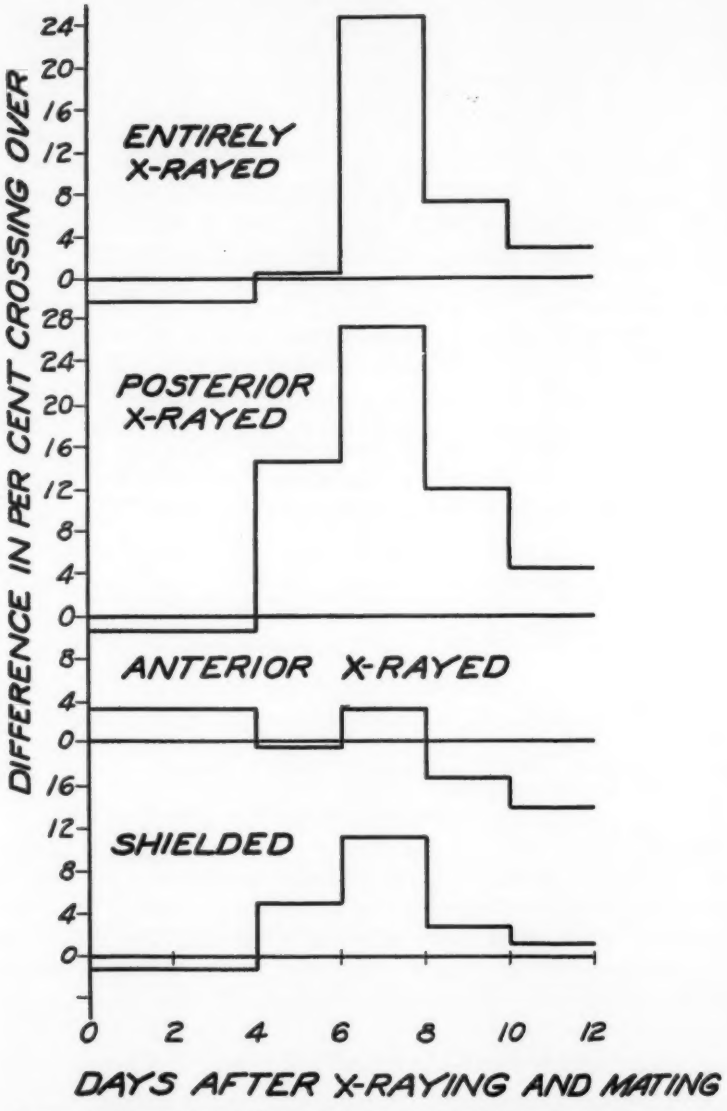

FIGURE 4.—Experiment 315 D. The ordinates indicate the differences in percent of crossing over between the treated and control flies, the abscissae indicate the days after X-ray treatment during which the eggs were laid from which the flies counted were hatched.

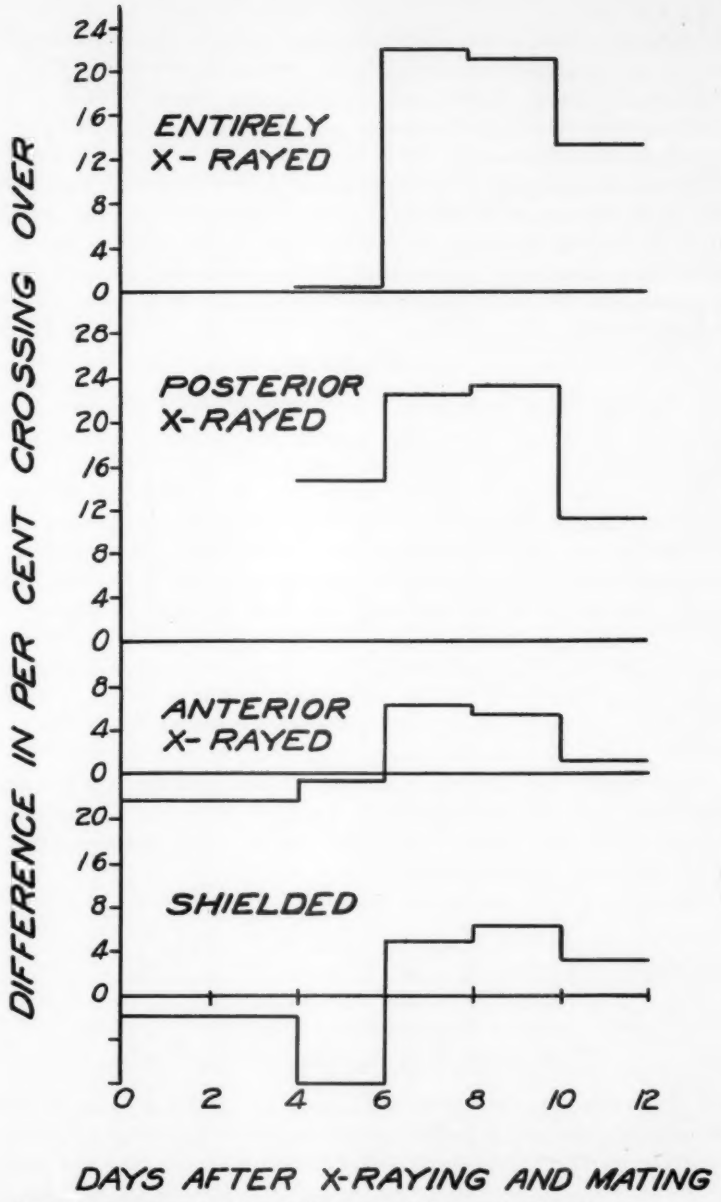

FIGURE 5.—Experiment 315 E. Ordinates and abscissae, as in Figure 4.

the crossover values in the x-chromosome. STERN (1926) has, however, recently shown an effect of age and temperature at the "bobbed," that is, "right" end of the sex chromosome. As this region is beyond the white to miniature region used in these experiments, STERN'S results have no direct bearing on this investigation. It is known, however, that in the case of XXY females in which secondary non-disjunction occurs the crossover value is slightly increased (BRIDGES 1916). Since, as noted later in this paper, exceptional offspring occurred in some of our cultures, this is a matter to be considered in comparing the crossover values found in the experiments, although, as explained under the data, it is quite unlikely that any secondary non-disjunction was involved in the actual experiments.

EXPERIMENT 315

In this experiment the X-rayed pupae were the female offspring of the mating of white-eyed, long-winged females with eosin-eyed, miniature-winged males. In preparing for the experiment there seemed to be a tendency in the stock to produce exceptions, although in all the pairs which were bred the percent of exceptions was too low to indicate secondary non-disjunction. That secondary non-disjunction did not occur in the experiment is shown by the counts of the offspring of the control females of the experiment which were sisters of the X-rayed females. The seven control females yielded a total of 3124 regular females, no exceptional females, 3217 regular males and two exceptional males: this is approximately the proportion in which exceptions have been found to occur normally in natural primary non-disjunction (BRIDGES 1916) (MAVOR 1924).

Female pupae for the experiment were taken from two matings designated *D* and *E*. Pair *D* produced 156 regular females, 146 regular males and one exceptional female. Pair *E* produced 154 regular females and 127 regular males and no exceptions.

Pupae from pair *D* were treated simultaneously as follows:

Anterior X-rayed, designated *Da*—8 pupae
Posterior X-rayed, designated *Db*—6 pupae
Control, designated *Dc*—7 pupae
Shielded, designated *Dd*—8 pupae
Entire pupa X-rayed, designated *De*—7 pupae

The X-ray treatment was given with the tube at 50 K.V. 5 M.A., with the pupae at a distance of 24 cm from the target, and lasted for 40 minutes. According to the method of expressing dosage used in a previous paper

(MAVOR 1924) this dose is represented by 35 *D*. After emergence the females from these pupae were mated to wild-type males.

The X-ray treatment was given on December 3, 1924. The flies were mated on December 4, transferred to second bottles on December 7, and to each of the third, fourth, and fifth bottles at two-day intervals, the transfers to each series of bottles being made simultaneously. The counts of the offspring are given in table 1, and shown graphically in figure 4.

TABLE 1

Experiment 315D. Counts of F_1 from X-rayed and Control pupae. The X-ray treatment was given at 2:15 P.M. December 3rd. The flies were mated at 6:00 P.M., December 4th, changed to second bottles on December 7th, third bottles on December 9th, fourth bottles on December 11th, fifth bottles on December 13th and killed on December 15th. Remaining therefore in the first bottles until four days after treatment, and in each of the second, third, fourth, and fifth bottles for two days.

BOTTLE	X-RAY TREATMENT	♀		♂					
		RED LONG	EXCEPTIONS	WHITE		EOSIN		EXCEPTIONS	PERCENT CROSSING OVER
				LONG	MIN.	LONG	MIN.		
1	Anterior	253	..	101	30	40	86	1	24.39
	Posterior	26	..	8	4	1	4	3	29.41
	Control	253	..	95	35	35	86	..	27.89
	Shielded	334	..	122	54	49	124	1	29.51
	Entire	18	..	4	1	3	2	1	40.00
2	Anterior	242	..	104	49	44	81	..	33.45
	Posterior	84	1	22	7	11	25	1	17.69
	Control	245	..	107	54	49	108	..	32.39
	Shielded	312	..	138	53	48	128	..	27.52
	Entire	78	..	18	8	10	21	2	31.58
3	Anterior	211	..	81	27	41	69	..	31.19
	Posterior	174	2	74	5	6	73	2	6.69
	Control	273	..	86	42	48	86	..	34.35
	Shielded	403	..	147	40	45	140	..	22.85
	Entire	135	..	71	4	10	60	3	9.66
4	Anterior	251	..	102	37	39	89	..	28.46
	Posterior	208	..	91	13	11	70	..	12.97
	Control	303	..	106	32	38	103	..	25.09
	Shielded	411	..	158	43	47	152	..	22.50
	Entire	231	3	84	18	20	93	..	17.67
5	Anterior	286	..	107	51	46	97	..	32.23
	Posterior	98	..	37	7	14	38	..	21.88
	Control	258	..	102	32	33	81	..	26.21
	Shielded	354	..	118	44	32	112	2	24.84
	Entire	97	..	44	9	14	32	..	23.23

Although when this part of the experiment is taken alone the numbers are rather small, certain effects of the X-rays are apparent. Firstly, the marked sterilizing effect of X-rays is evident in the case of pupae in which the posterior half or the entire body has been exposed, there being, however, practically no difference in the fertility between pupae exposed over their anterior half, shielded but submitted to an amount of secondary radiation equal to that received by the posterior half when the anterior is X-rayed, and pupae not in any way exposed to X-rays, excepting that the fertility of the shielded pupae was slightly the greater. The numbers in the case of the pupae exposed either entirely or in the posterior region are too small to give crossover values with certainty. There is, however, a conspicuous decrease in the crossover value only in the case of these pupae.

Pupae from pair *E* were treated simultaneously as follows:

Anterior X-rayed, *Ea*—5 pupae
Posterior X-rayed, *Eb*—4 pupae
Control, *Ec*—5 pupae
Shielded, *Ed*—4 pupae
Entirely X-rayed, *Ee*—4 pupae

The X-ray treatment was exactly the same as in the case of the pupae of 315 D. The counts of the offspring are given in table 2 and represented graphically in figure 5. This table shows the same features as table 1 and the results of this latter part of the experiment agree precisely with those of the first part.

The data from the two parts of the experiment are combined in table 3 and represented graphically in figure 6. The probable errors in this table, as in other tables in this paper, were obtained by comparing the counts of the different categories of treated flies with the counts of the control flies, using the well known formula due to K. PEARSON. The crossover values for the control flies were not the same in each of the five bottles, the values in the second and third bottles being slightly higher than those in the preceding or subsequent bottles. It is hoped to make the subject of the variation of crossing over between the loci of white-eye color and miniature wing the subject of a subsequent paper. It need only be remarked that the variation in crossing over in the control, while it may be significant, does not affect the general results of these experiments.

The two categories of treated pupae which show the greatest modification of the crossover value are those in which the posterior half and the entire animal were exposed. There is, however, a striking difference between the two cases. The counts from the first bottles of these flies were too small

to give a significant crossover value. From our previous work it may, however, be assumed to be normal. In the second bottles, however, (eggs laid during the fifth and sixth days after X-ray treatment) while the entirely

TABLE 2

Experiment 315E. Counts of F_1 from X-rayed and Control pupae. The X-ray treatment was given at 4:40 P.M. December 3rd. The flies were mated at 4:30 P.M. on December 5th, changed to second bottles on December 7th, to third bottles on December 9th, to fourth bottles on December 11th, to fifth bottles on December 13th, and killed on December 15th. Remaining therefore in the first bottles until four days after treatment and in each of the second, third, fourth, fifth bottles for two days.

BOTTLE	X-RAY TREATMENT	♀		♂					
		RED LONG	EXCEPTIONS	WHITE		EOSIN		EXCEPTIONS	PERCENT CROSSING OVER
				LONG	MIN.	LONG	MIN.		
1	Anterior	347	..	144	43	33	108	..	23.17
	Posterior	6	..	1	..	..	1	..	..
	Control	340	..	126	37	26	110	..	21.07
	Shielded	251	..	105	22	30	70	..	22.91
	Entire	1	..	2	2	..	..	1	..
2	Anterior	351	..	172	54	75	134	..	29.66
	Posterior	37	..	11	3	..	8	2	13.64
	Control	276	..	123	50	47	112	1	29.22
	Shielded	278	1	96	47	59	82	1	37.32
	Entire	34	..	6	4	4	14	2	28.57
3	Anterior	340	..	157	38	44	131	1	22.16
	Posterior	81	..	22	2	3	47	1	5.95
	Control	361	..	133	45	67	153	..	28.39
	Shielded	270	..	120	31	36	95	1	23.75
	Entire	45	1	19	1	2	27	1	6.12
4	Anterior	303	..	170	48	28	115	..	21.05
	Posterior	120	..	60	2	3	60	1	4.00
	Control	385	..	165	56	57	143	..	26.84
	Shielded	230	1	120	29	33	119	..	20.60
	Entire	118	2	68	5	3	63	..	5.76
5	Anterior	418	..	168	51	45	136	1	24.00
	Posterior	60	..	30	3	6	23	..	14.52
	Control	430	..	151	60	44	154	1	25.43
	Shielded	273	..	120	32	35	118	2	21.97
	Entire	176	..	80	10	8	52	1	12.00

X-rayed flies show an approximately normal crossover value, practically identical with that of the control flies, the flies which were X-rayed only in the posterior half show a very noticeable and significant decrease in the crossover value to 12.14. In the third bottles the decrease in the crossover

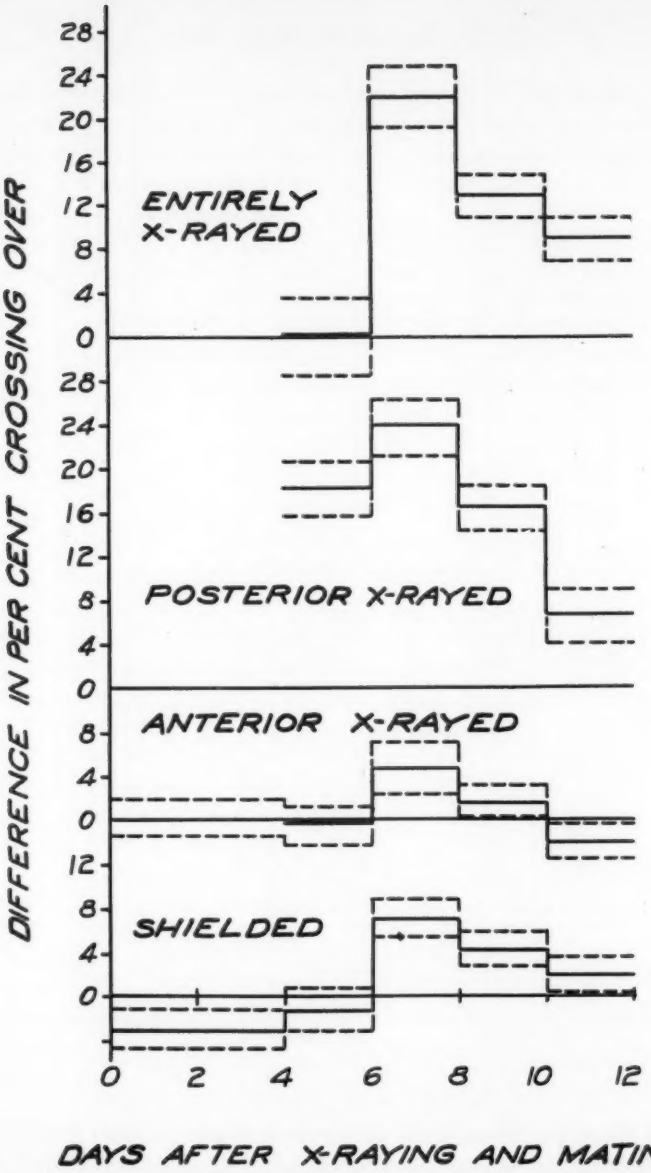

FIGURE 6.—Experiment 315 combined results. The ordinates indicate the difference in percent of crossing over between the treated and control flies: the abscissae the days after the X-ray treatment during which the eggs were laid from which the flies counted were hatched. The continuous lines give the actual values obtained for the differences, and the interrupted lines the differences plus or minus the probable errors of the differences.

values is even greater, being for the entirely X-rayed flies 8.76 and for the flies X-rayed in the posterior half 6.61. A subsequent recovery toward the normal crossover value is evident in both cases in the fourth and fifth bottles.

TABLE 3

Experiment 315. Data from parts D and E combined.

BOTTLE	X-RAY TREATMENT	TOTAL REGULAR F		CROSSING OVER			EXCEPTIONS	
				PERCENT	COMPARED WITH CONTROL		PERCENT	PERCENT
		♀	♂		P. E. DIFF IN PERCENT	DIFF. P. E. DIFF.	♀	♂
1	Anterior	600	61	23.74	1.69	.26	0.0	0.16
	Posterior	32	19	26.32	..	..	0.0	15.79
	Control	593	550	24.18	..	..	0.0	0.0
	Shielded	585	576	26.91	1.72	1.59	0.0	0.17
	Entire	19	14	42.86	..	..	0.0	14.29
2	Anterior	593	713	31.14	1.69	.22	0.0	0.0
	Posterior	121	173	12.14	2.66	7.00	0.83	1.73
	Control	521	650	30.77	..	..	0.0	0.15
	Shielded	590	651	31.80	1.73	.60	0.17	0.15
	Entire	112	85	30.59	..	..	0.0	4.71
3	Anterior	551	588	25.51	1.79	2.93	0.0	0.17
	Posterior	255	242	6.61	2.34	10.32	0.78	1.24
	Control	634	660	30.76	..	..	0.0	0.0
	Shielded	673	654	23.24	1.72	4.38	0.0	0.15
	Entire	180	194	8.76	2.54	8.65	0.56	2.06
4	Anterior	554	628	24.20	1.63	1.19	0.0	0.0
	Posterior	328	310	9.35	2.02	8.31	0.0	0.32
	Control	688	700	26.14	..	..	0.0	0.0
	Shielded	641	701	21.68	1.58	2.82	0.16	0.0
	Entire	349	354	12.99	1.93	.68	1.43	0.0
5	Anterior	704	701	27.53	1.60	1.13	0.0	0.14
	Posterior	158	158	18.99	2.61	2.58	0.0	0.0
	Control	688	657	25.72	..	..	0.0	0.15
	Shielded	627	611	23.40	1.66	1.40	0.0	0.65
	Entire	273	249	16.47	2.20	4.20	0.0	0.40

Attention may now be turned to the counts from flies emerging from pupae X-rayed in the anterior half and shielded, that is, pupae not exposed to direct radiation but placed so as to receive approximately the same amount of secondary radiation as the posterior half of pupae which were exposed in the anterior half. The counts from the anteriorly X-rayed pupae show some decrease in the crossover value in the third and fourth bottles

(seventh to tenth days after treatment); this is, however, shown in the table to be of no statistical significance. The counts from the shielded pupa show a similar decrease in the third and fourth bottles which may, however, be of statistical significance in the case of the third bottles; $\frac{\text{Diff.}}{\text{P.E.diff.}} = 4.38$.

EXPERIMENT 316

In this experiment the pupae which were X-rayed were of the same genetic constitution as those in Experiment 315, being $\frac{w+}{w^e m}$ and were obtained from the mating of white-eyed females with eosin, miniature males; they were, however, from one to three days younger when treated than the pupae of that experiment. To obtain pupae for this experiment six matings, *A* to *F*, were made and yielded a total of 796 regular females, 1 exceptional female, 702 regular males and 3 exceptional males, showing as in the previous experiment a tendency to produce exceptions. Female pupae were taken from pairs *B* and *D* for X-ray treatment. Pair *B* produced 237 regular females, 178 regular males and no exceptions. Pair *D* produced 155 regular females, 167 regular males and no exceptions.

Pupae from *B* were treated in two groups. In Group I the numbers of pupae were,

Anterior	3
Posterior	3
Control	3
Shielded	3
Entire	3

and in Group II

Anterior	4
Posterior	4
Control	4
Shielded	4
Entire	4

The X-ray treatment was given with the Coolidge tube at 50.000 volts, 5.0 M.A., distance 17.5 cm and lasted for 18 minutes. This dose is slightly less than that given in Experiment 315, namely 30 *D*. After emergence the flies were mated to wild-type males. The pupae of Group I were treated on February 15 at 5.00 P.M., mated on February 17 and transferred to second, third, fourth, and fifth bottles on February 19, 21, 23, and 25

respectively. The parents were killed on February 27. The pupae of Group II were treated on February 16 at 12.30 P.M., mated February 18 and transferred to second, third, fourth, and fifth bottles on February 20, 22,

TABLE 4

Experiment 316B. Counts of F_1 from X-rayed and Control pupae. The data from the two groups of pupae, No. 1 and 2 have been combined in the table. The details of X-ray treatment and mating are given in the text. The parents remained until four days after treatment in the first bottles and two days in each of the other bottles.

BOTTLE	X-RAY TREATMENT	♀						♂	
		RED LONG	EXCEPTIONS	WHITE		EOSIN		EXCEPTIONS	PERCENT CROSSING OVER
				LONG	MIN.	LONG	MIN.		
1	Anterior	434	..	135	61	45	160	1	26.43
	Posterior	5	..	1	..	1	1	..	33.33
	Control	675	..	244	83	77	291	..	23.02
	Shielded	356	..	167	44	40	133	1	21.88
	Entire	4	..	1	..	..	1	..	..
2	Anterior	323	..	122	48	45	109	2	28.70
	Posterior	5	..	..	..	..	1	..	..
	Control	628	..	221	92	112	229	2	31.19
	Shielded	248	..	106	32	38	91	1	26.22
	Entire	6	..	5	..	..	1	..	..
3	Anterior	381	..	162	59	46	139	..	25.86
	Posterior	27	..	5	1	..	9	..	6.67
	Control	723	..	301	105	117	280	..	27.65
	Shielded	241	..	99	32	39	84	..	27.95
	Entire	19	..	9	1	1	11	..	4.88
4	Anterior	387	..	178	37	33	159	..	17.20
	Posterior	24	..	7	2	1	7	..	17.65
	Control	718	1	284	63	83	247	..	21.57
	Shielded	298	..	114	32	24	110	..	20.00
	Entire	28	..	5	1	1	6	..	15.38
5	Anterior	351	..	150	35	24	145	..	16.67
	Posterior	25	..	12	2	..	3	..	11.76
	Control	702	2	301	77	102	266	1	23.99
	Shielded	297	..	130	34	28	135	..	18.96
	Entire	42	..	23	3	3	18	..	12.76

24, and 26 respectively. The parents were killed on February 28. In each case the transfers to new bottles were made at approximately the same time of day as the X-ray treatment occurred. The counts of the offspring in this part of the experiment are given in table 4, and shown graphically in figure 7.

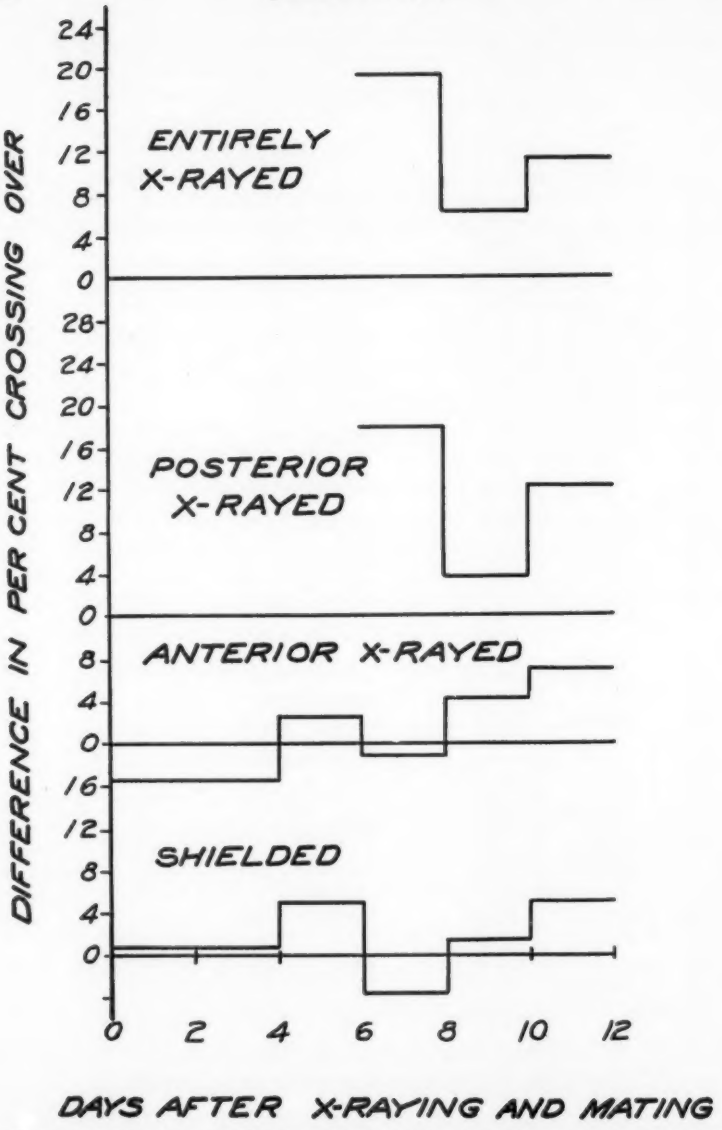

FIGURE 7.—Experiment 316 B. The ordinates indicate the difference in percent of crossing over between the treated and control flies. The abscissae indicate the days after X-ray treatment during which the eggs were laid from which the flies counted were hatched.

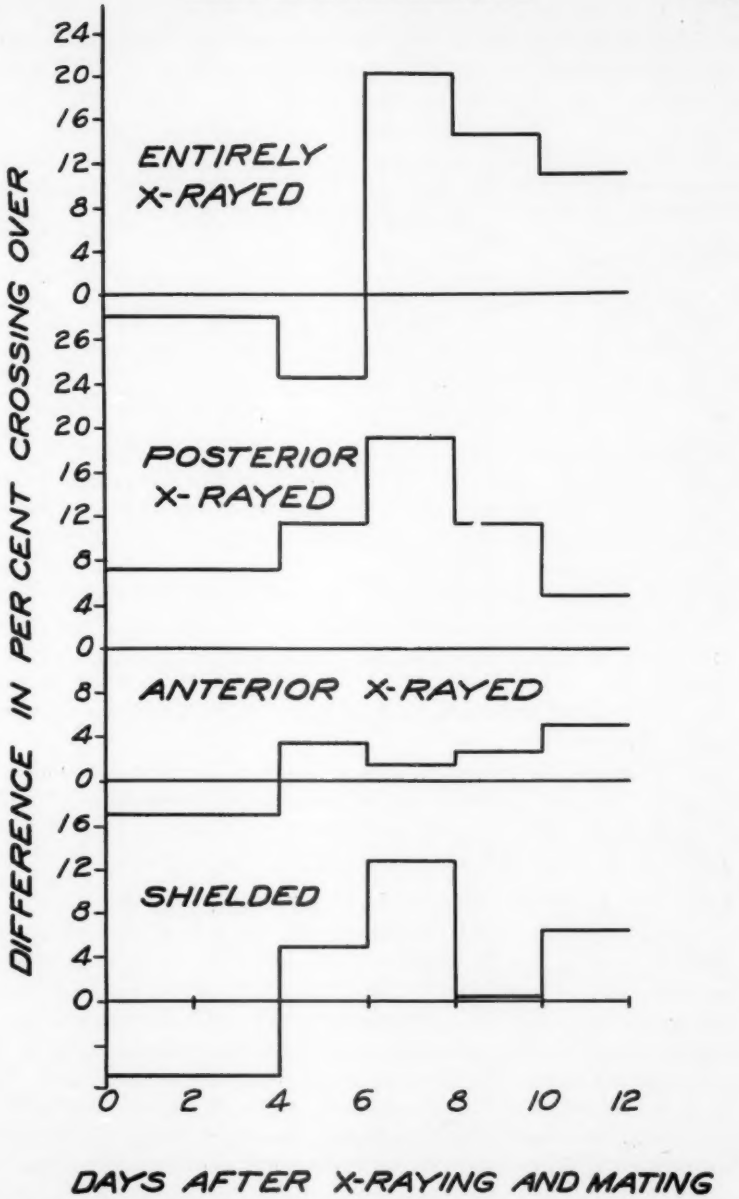

FIGURE 8.—Experiment 316 D. The ordinates and abscissae are the same as in Figure 7.

Pupae from *D* were treated in two groups. The numbers of pupae treated in Group I were,

Anterior	4
Posterior	5
Control	1
Shielded	3
Entire	5

and in Group II,

Anterior	1
Posterior	2
Control	8
Shielded	2
Entire	2

The X-ray treatment of the pupae of *D* was the same as that of *B*. The pupae of Group I were treated on February 15 at 6.30 P.M. and mated on February 18 with the exception of the shielded pupae which did not all emerge until February 19 and were mated on that day. As in Experiment 315, four days after treatment, the flies were transferred to second bottles and after that to new bottles at two-day intervals until they had passed through five bottles. The pupae of Group II were treated on February 16 simultaneously with those of *B*, Group II, mated on February 19 and transferred to new bottles at two day intervals, passing through five bottles. The data from the two groups of *D* are given in table 5 and shown graphically in figure 8.

The data from the two parts of the experiment, *B* and *D*, are combined in table 6 and the difference in the crossover values together with the probable errors of the differences are shown graphically in figure 9. The results of this experiment are in accord with the previous experiment.

COMBINED RESULTS OF EXPERIMENTS 315 AND 316

The technique and procedure was exactly the same in the two experiments, excepting that the pupae of Experiment 316 were X-rayed at a somewhat earlier stage than in Experiment 315. If the graphs of these two experiments (figures 6 and 9) be compared it will be noticed that there appears to have been a greater effect produced by the treatment given in a later pupal stage. The X-ray treatment was not, however, quite the same in the two cases. In Experiment 315 in which older pupae were treated, the dose was 35 *D*, while in Experiment 316 in which younger pupae were treated the dose was 30 *D*. This difference in the crossover value be-

tween the two experiments, apparent only in the case of pupae treated entirely and in the posterior region, is, however, not greater than the probable error.

TABLE 5

Experiment 315D. Counts of F_1 from X-rayed and Control pupae. The data from the two droups of pupae, No. I and II have been combined in the table. The details of X-ray treatment and mating are given in the text. The parents remained until four days after treatment in the first bottles and two days in each of the other bottles.

BOTTLE	X-RAY TREATMENT	♀		♂					
		RED LONG	EXCEPTIONS	WHITE		EOSIN		EXCEPTIONS	PERCENT CROSSING OVER
				LONG	MIN.	LONG	MIN.		
1	Anterior	230	..	71	24	25	89	..	23.44
	Posterior	28	..	5	1	2	15	..	13.04
	Control	420	2	163	40	46	174	2	20.33
	Shielded	117	..	48	14	16	34	..	26.79
	Entire	13	..	3	2	..	4	1	22.22
2	Anterior	226	..	89	22	38	100	1	24.10
	Posterior	21	..	10	2	1	6	1	15.79
	Control	380	..	135	43	47	100	1	27.61
	Shielded	248	..	125	27	38	97	..	22.65
	Entire	23	..	6	5	1	5	..	35.29
3	Anterior	279	..	109	52	32	96	..	29.07
	Posterior	127	1	41	4	9	58	3	11.61
	Control	475	..	188	79	86	181	2	30.90
	Shielded	303	..	109	36	36	109	..	18.46
	Entire	73	..	44	3	6	31	..	10.71
4	Anterior	299	1	110	31	40	127	..	23.05
	Posterior	121	..	47	4	13	56	..	14.17
	Control	594	..	215	72	73	207	1	25.57
	Shielded	383	..	145	41	54	132	..	25.54
	Entire	112	..	45	6	2	40	..	10.53
5	Anterior	315	..	130	27	34	118	..	19.74
	Posterior	128	..	42	12	11	49	1	20.18
	Control	491	..	195	63	60	177	..	24.85
	Shielded	340	..	140	26	38	139	..	18.66
	Entire	127	..	51	9	6	47	..	13.27

Setting this difference aside, it is legitimate to combine the results of the two experiments by adding the counts of the corresponding bottles. When this is done table 7 is obtained, the results of which are shown graphically in figure 10. The forms of the graphs, as might be expected, are approximately the same as those for the individual experiments; the prob-

able errors being smaller, the graphs are more uniform and show somewhat more clearly the features referred to in considering the graphs of Experiment 315.

TABLE 6

Experiment 316. Data from parts B and D combined.

BOTTLE	X-RAY TREATMENT	TOTAL REGULAR F.		CROSSING OVER			EXCEPTIONS	
				PERCENT	COMPARED WITH CONTROL		PERCENT	PERCENT
		♀	♂		P. E. DIFF IN PERCENT	DIFF. P. E. DIFF.	♀	♂
1	Anterior	664	610	25.41	1.41	2.42	0.0	0.16
	Posterior	33	26	15.38	..	..	0.0	0.0
	Control	1095	1118	22.00	..	..	0.18	0.18
	Shielded	473	496	22.98	1.51	.65	0.0	0.20
	Entire	17	11	18.18	..	..	0.0	9.09
2	Anterior	549	573	26.70	1.63	2.04	0.00	0.52
	Posterior	26	20	15.00	..	..	0.0	5.0
	Control	1008	979	30.03	..	..	0.0	0.31
	Shielded	496	554	24.37	1.64	3.44	0.0	0.18
	Entire	29	23	26.10			0.0	0.0
3	Anterior	660	695	27.19	1.46	1.21	0.0	0.0
	Posterior	154	127	11.02	2.84	6.31	0.69	2.36
	Control	1198	1337	28.95	..	..	0.0	0.15
	Shielded	544	644	22.20	1.47	4.60	0.0	0.0
	Entire	92	106	10.38	3.54	5.23	0.0	0.0
4	Anterior	686	715	19.72	1.34	1.74	0.15	0.0
	Posterior	145	137	14.60	2.57	3.41	0.0	0.0
	Control	1312	1244	23.39	..	..	0.08	0.08
	Shielded	681	652	23.16	1.38	.17	0.0	0.0
	Entire	140	108	11.11	2.86	4.28	0.0	0.0
5	Anterior	666	663	18.10	1.39	4.48	0.0	0.15
	Posterior	153	131	19.08	2.66	1.98	0.0	0.76
	Control	1193	1241	24.34	..	..	0.17	0.08
	Shielded	637	670	18.81	1.39	3.99	0.0	0.0
	Entire	169	160	13.13	2.43	4.61	0.0	0.0

Unfortunately the sterilizing effect of X-ray exposure of the entire pupa or its posterior part is so great during the first four days after the treatment that no significant value could be found for the crossing over of eggs maturing in that time. In the case of the entirely X-rayed pupae the crossover value was unaffected during the fifth and sixth days after treatment, while in the case of pupae exposed only in the posterior half the crossover value, as shown by counts from eggs laid on the fifth and sixth days after treat-

ment, was significantly decreased. For the second bottles (eggs laid on the fifth and sixth days after treatment) the difference between the crossover values of the entirely and posteriorly X-rayed pupae is 4.6 times the probable error of the difference. For the third bottles (eggs laid on the

TABLE 7

Experiments 315-316. Data from the two experiments combined.

BOTTLE	X-RAY TREATMENT	TOTAL REGULAR		CROSSING OVER			EXCEPTIONS	
				PERCENT	COMPARED WITH CONTROL		PERCENT	PERCENT
		♀	♂		P. E. DIFF. IN PERCENT	DIFF. P. E. DIFF.	♀	♂
1	Anterior	1264	1225	24.57	1.06	1.74	0.0	0.16
	Posterior	65	45	20.00	..	..	0.0	6.67
	Control	1688	1668	22.72	..	..	0.12	0.12
	Shielded	1058	1072	25.09	1.11	2.14	0.0	0.19
	Entire	36	25	32.00	..	..	0.0	12.00
2	Anterior	1142	1286	29.16	1.16	1.01	0.0	0.23
	Posterior	147	193	12.44	2.36	7.78	0.68	2.07
	Control	1529	1629	30.33	..	..	0.0	0.25
	Shielded	1086	1205	28.38	1.18	1.66	0.09	0.17
	Entire	141	108	29.63	3.08	.23	0.0	3.70
3	Anterior	1211	1283	26.42	2.00	1.56	0.0	0.08
	Posterior	409	369	8.13	1.75	12.26	0.73	1.63
	Control	1832	1997	29.54	..	..	0.0	0.10
	Shielded	1217	1298	22.73	1.10	6.21	0.0	0.08
	Entire	272	300	9.33	1.91	10.59	0.37	1.33
4	Anterior	1240	1343	21.82	1.03	2.49	0.08	0.0
	Posterior	473	447	10.96	1.52	8.83	0.0	0.22
	Control	2000	1944	24.38	..	..	0.05	0.05
	Shielded	1322	1353	22.39	1.03	1.94	0.08	0.0
	Entire	489	462	12.55	1.50	7.89	1.02	0.0
5	Anterior	1370	1364	22.95	1.03	1.81	0.0	0.15
	Posterior	311	289	19.03	1.84	3.15	0.0	0.35
	Control	1881	1898	24.82	..	..	0.11	0.11
	Shielded	1264	1281	21.00	1.05	3.63	0.0	0.31
	Entire	442	409	15.16	1.59	6.08	0.0	0.24

seventh and eighth days after treatment) the crossover values of the entirely and posteriorly X-rayed pupae are approximately the same, the crossover value being in each case decreased from approximately 30 to approximately 9 and 8, the greatest decrease being in the case of the pupae X-rayed in the posterior half. In the fourth and fifth bottles (eggs laid during the ninth and twelfth days after X-ray treatment) there is, as in

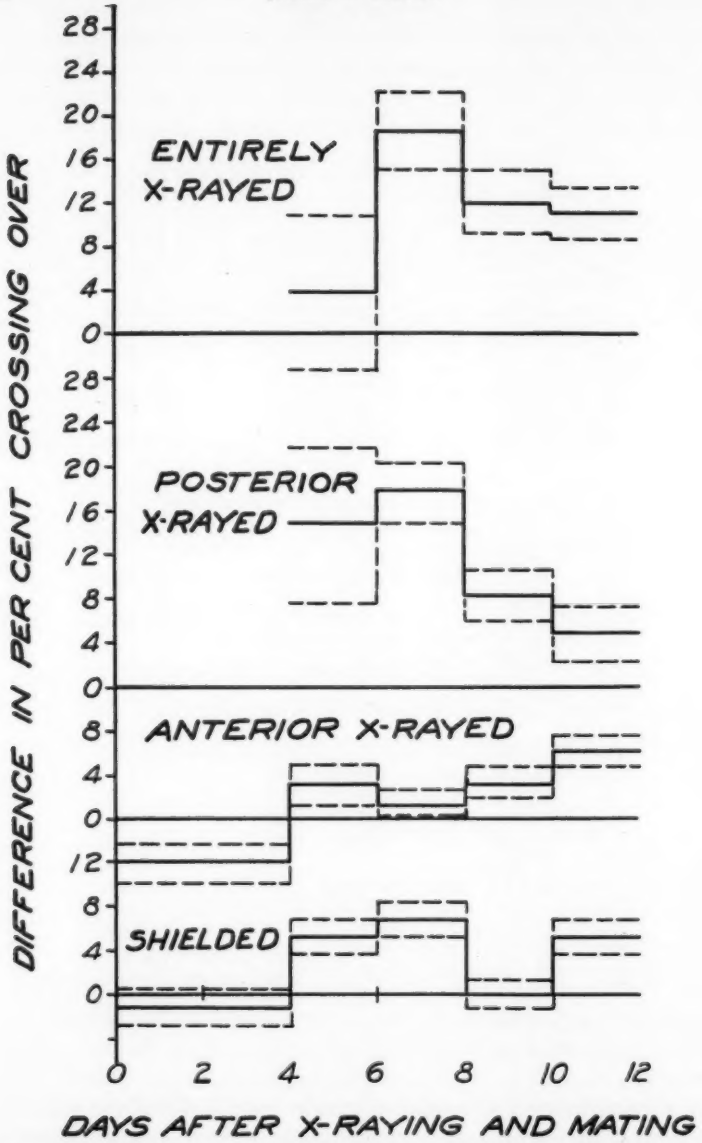

FIGURE 9.—Experiment 316 combined results. The ordinates indicate the difference in percent of crossing over between the treated and control flies, the abscissae the days after X-ray treatment during which the eggs were laid from which the flies counted were hatched. The continuous lines give the actual values obtained for the differences and the interrupted lines the differences plus or minus the probable errors of the differences.

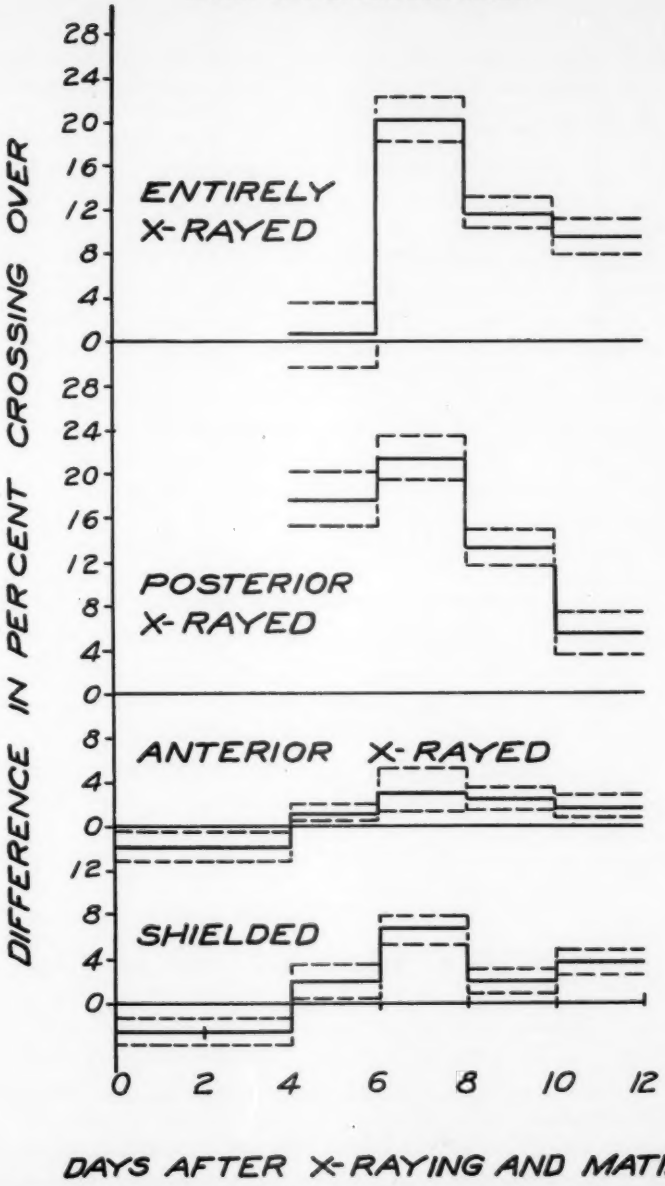

FIGURE 10.—Experiments 315 and 316 combined results. The ordinates indicate the difference in percent of crossing over between the treated and control flies, the abscissas the days after X-ray treatment during which the eggs were laid from which the flies counted were hatched. The continuous lines give the actual values obtained for the differences and the interrupted lines the differences plus and minus the probable errors of the differences.

the writer's previous experiments (MAVOR 1923) a gradual recovery toward the normal crossover value, the recovery being, however, apparently

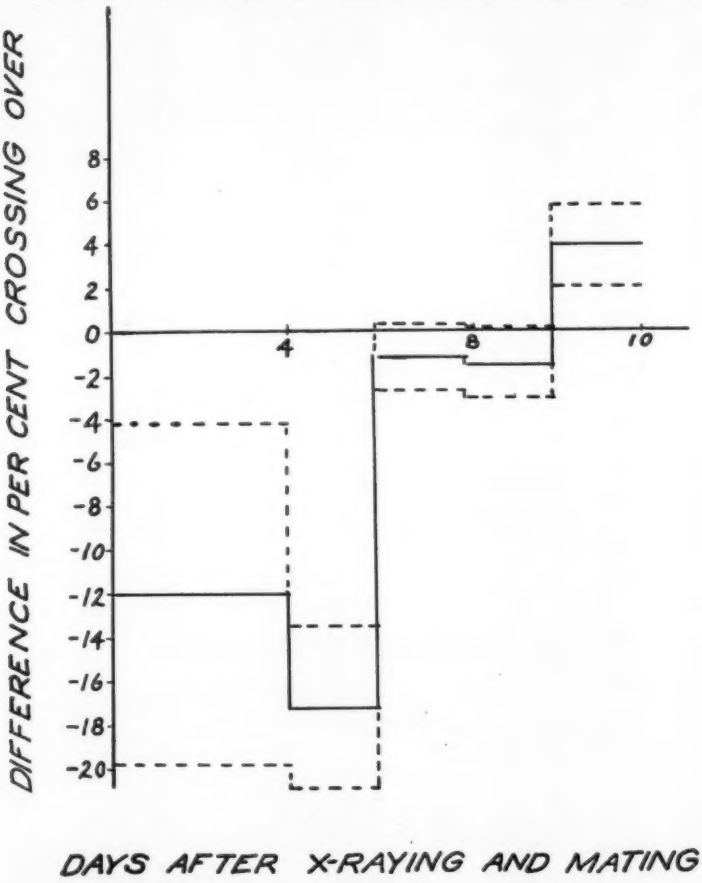

FIGURE 11.—Experiments 315 and 316. Comparison of crossing over in pupae X-rayed entirely and in the posterior half. The ordinates indicate the differences in percent crossing over, posterior minus entire, and the abscissae the days after X-ray treatment during which the eggs were laid from which flies counted were hatched. The continuous lines give the actual values obtained for the differences and the interrupted lines the differences plus and minus the probable errors of the differences.

more rapid in the case of the pupae X-rayed in the posterior region. The results of X-raying the posterior half and the entire animal are compared

graphically in figure 11. The graph shows the differences, posterior minus entire, continuous line, and the probable errors of these differences, interrupted lines, for each of the intervals. This difference may be significant in the second bottles, fifth and sixth days.

On turning to a consideration of crossing over in the pupae treated in the anterior half, it is clearly seen that at no time was there any significant modification of the crossover value. In the case of the shielded pupae there may have been a slight decrease in the crossover value in the third bottles (eggs laid during the seventh and eighth days after X-ray treatment). A comparison of the counts of the third bottles of the anteriorly X-rayed and the shielded pupae shows that the difference in the crossover values is 3.15 times the probable error of the difference and may be significant.

Although this paper deals primarily with crossing over, it will be of interest to mention the occurrence of exceptions. The data is scarcely extensive enough to lend itself to statistical treatment. It is clear, however, that the only pupae in which non-disjunction was induced by the X-ray treatment were those which were either entirely X-rayed or those which were treated in the posterior half. The percent of exceptions occurring in these is comparable to that found by the writer in previous experiments (MAVOR 1924). The percent of exceptions is greater in the case of pupae entirely exposed to X-rays than in the case of pupae exposed only in the posterior half.

EXPERIMENT 320

The technique of this experiment was identical with that of the two experiments already described. The X-ray dose was 35 *D* (50 K.V. 5 M.A. 16.8 cm. 28.2 min.). The pupae were treated not more than twenty-four hours before emergence. After emergence the females, which were $\frac{w+}{w^e m}$, were mated to wild-type males. The pairs remained in the first bottles until four days after the X-ray treatment and in each of the subsequent bottles for two days. The results of the experiment are shown in tables 8 and 9.

The results of this experiment are in agreement with those of the previous experiments, Nos. 315 and 316. The number of offspring counted in determining the crossover values was large, a total of over 10,000 male F_1 males having been classified. The number of F_1 counted for each egg-laying period, (that is, "bottle") for the anteriorly X-rayed, posteriorly X-rayed and control was correspondingly large, ranging from 600 to 900.

These numbers ensure a relatively accurate determination of the crossover values, and nowhere is the probable error in percent of the difference between either the anteriorly X-rayed or the shielded and the control greater than 1.7. Also, nowhere is the difference between the anteriorly X-rayed

TABLE 8

Experiment 320. Counts of F_1 from X-rayed and control pupae. The X-ray dose was $35D_{10}$ (50 KV, 5 M. A. 16.8 cm. 28.2 min.) The pairs remained in the first bottles until 4 days after the treatment, and in each of the others for 2 days.

BOTTLE	X-RAY TREATMENT	♀		♂					
		RED LONG	EXCEPTIONS	WHITE		EOSIN		EXCEPTIONS	PERCENT CROSSING OVER
				LONG	MIN.	LONG	MIN.		
1st.	Anterior	661	..	264	110	111	241	..	30.44
	Posterior	5	..	1	..	..	..	..	..
	Control	907	..	299	125	118	298	2	28.93
	Shielded	631	..	245	85	99	266	1	16.47
	Entire	73	..	34	11	8	23	1	24.68
2nd.	Anterior	635	..	207	104	111	212	..	33.91
	Posterior	6	..	2	1	1	4	..	..
	Control	897	..	288	158	147	302	..	34.08
	Shielded	644	..	238	96	88	163	..	31.45
	Entire	2	..	2	..	..	2	..	..
3rd.	Anterior	623	..	242	95	78	220	3	27.56
	Posterior	44	..	12	5	2	25	..	15.91
	Control	736	..	283	121	104	319	1	27.21
	Shielded	597	..	234	90	79	214	1	27.39
	Entire	108	..	40	8	6	50	..	13.46
4th.	Anterior	654	1	235	99	94	223	..	29.65
	Posterior	65	..	28	1	1	21	..	3.92
	Control	828	..	261	118	110	289	1	29.31
	Shielded	663	1	220	101	106	217	1	32.14
	Entire	131	..	60	9	7	55	1	12.21
5th.	Anterior	648	..	228	97	102	225	1	30.52
	Posterior	25	..	8	..	2	14	..	8.33
	Control	727	..	262	132	119	246	..	33.07
	Shielded	687	..	238	98	122	250	..	31.07
	Entire	117	..	41	17	21	57	..	27.94

and the control or the shielded and the control as much as twice this probable error. The experiment is therefore fairly conclusive regarding the question which the investigation was undertaken to answer, namely whether a treatment not involving the direct radiation of the ovary could affect the crossover value. Regarding, however, the question raised by

the results of the two previous experiments as to whether any difference could be recognized in crossover value between pupae entirely X-rayed and pupae X-rayed only in the posterior half the numbers of F_1 are still too few to give a positive answer.

TABLE 9

Experiment 320. Difference, control-treated, between the crossover values determined from the control and the treated females. X-ray dose was 35D_{50} (50 KV, 5 MA, 16.8 cm. 28.2 min.). The pairs remained in the first bottles until 4 days after the treatment and in each of the other bottles for 2 days.

BOTTLE	TREATMENT	CROSSING OVER			
		PERCENT	DIFFERENCE CONTROL-TREATED	P. E. DIFF. IN PERCENT	DIFF. P. E. DIFF.
1	Anterior	30.44	− 1.51	1.55	.97
	Posterior	..	..		
	Control	28.93			
	Shielded	26.47	+ 2.46	1.57	1.57
	Entire	24.68	+ 4.25	3.64	1.17
2	Anterior	33.91	+ .17	1.66	.10
	Posterior	..	..		
	Control	34.08			
	Shielded	31.45	2.63	1.70	1.55
	Entire	..	..		
3	Anterior	27.56	− .35	1.59	.22
	Posterior	15.91	+11.30	4.64	2.44
	Control	27.21			
	Shielded	27.39	− .18	1.60	.11
	Entire	13.46	+13.75	3.12	4.41
4	Anterior	29.65	− .34	1.63	.21
	Posterior	3.92	+25.39	4.45	5.71
	Control	29.31			
	Shielded	32.14	− 2.83	1.64	1.73
	Entire	12.21	+17.10	2.90	5.90
5	Anterior	30.52	+ 2.55	1.69	1.51
	Posterior	8.33	+24.74	6.97	3.55
	Control	33.07			
	Shielded	31.07	+ 2.00	1.66	1.20
	Entire	27.94	+ 5.13	2.95	1.74

EXPERIMENT 322

Since the previous experiment, number 320, seemed to show conclusively no appreciable difference in the results of treating the anterior half only and shielding the entire pupa so that it received approximately the same amount of secondary radiation as the posterior half of the pupa which

was X-rayed anteriorly, in the next experiment attention was concentrated on the effects of X-raying the entire pupa and its posterior half only. The object of this experiment, number 322, was to compare as accurately as possible the effects of X-raying pupae entirely and in the posterior half only. No pupae, therefore, were treated in the anterior half or shielded.

As in the previous experiments the genetic constitution of the control and X-rayed pupae was $\frac{w+}{w^e m}$. The experiment was performed at the MARINE BIOLOGICAL LABORATORY, Woods Hole, and the X-ray treatment was given with the Snook equipment of that laboratory. The voltage was determined by the spark gap between spheres and was maintained at 65 K.V. Peak., which was considered to be approximately equal to 50 K.V. root mean square, the voltage used in the previous experiments. The treatment was given at 5 M.A. and 23 centimeter distance, and for a period of 31 minutes 44 seconds; according to our method of indicating dosage this is indicated by 30 *D*.

The technique subsequent to the X-ray treatment was the same as in the previous experiments, the flies which emerged being mated in separate bottles. They remained in the first bottles until four days after the X-ray

TABLE 10

Experiment 322. First part. (parts 1,3,5). Counts of F_1 from X-rayed and control pupae. The X-ray dose was $30D_{50}$ [50 KV (65 KV Peak) 5 M.A. 23 cm. 31 min. 44 sec.] The pairs remained in the first bottles until 4 days after the treatment, and in each of the other bottles for 2 days.

BOTTLE	TREATMENT	♀		♂						
				WHITE		EOSIN		EXCEPTIONS	PERCENT	
		RED LONG	EXCEPTIONS	LONG	MIN.	LONG	MIN.		CROSSING OVER	EXCEPTIONS
1	Entire	390	1	134	52	59	118	8	30.6	2.16
	Posterior	430	..	152	72	58	98	14	34.2	3.56
	Control	539	..	199	66	78	188	..	27.1	0.00
2	Entire	626	..	250	41	36	275	5	12.8	0.82
	Posterior	658	..	293	54	45	288	5	14.6	0.73
	Control	534	..	166	66	82	197	1	29.0	0.20
3	Entire	756	2	307	42	40	314	2	11.7	0.28
	Posterior	848	1	348	57	62	375	..	14.1	0.00
	Control	605	..	206	69	70	231	..	24.1	0.00
4	Entire	771	..	319	79	88	286	1	21.6	0.13
	Posterior	788	1	333	57	90	308	2	18.7	0.26
	Control	578	..	225	86	98	233	..	28.7	0.00

treatment and were subsequently transferred to new bottles at intervals of two days. The incubator was maintained at a temperature of 25° C.

In the first part of the experiment female pupae were treated as follows:

X-rayed entirely	16
X-rayed in posterior half	16
Control	8

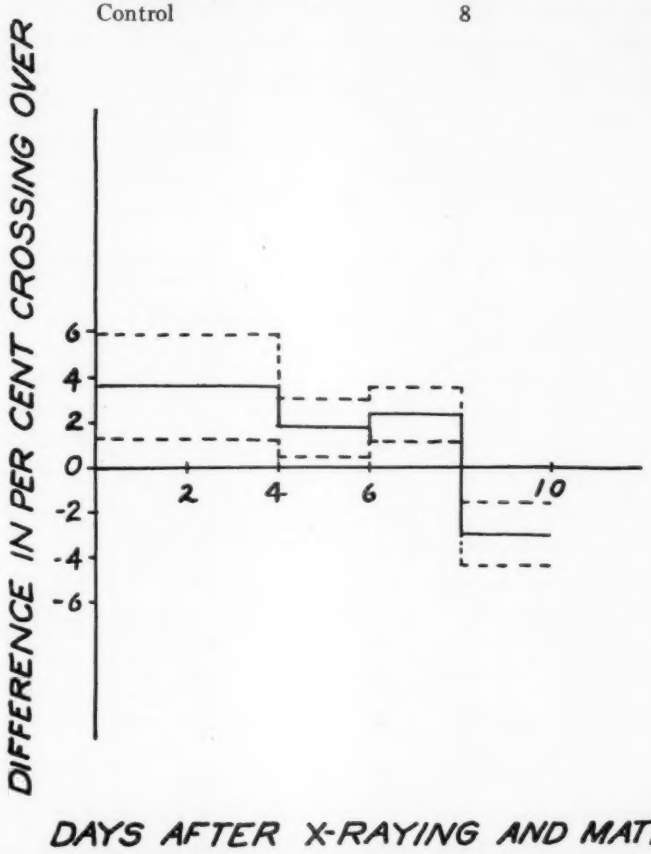

FIGURE 12.—Experiment 322. Comparison of crossing over in pupae X-rayed in the posterior half and over the entire body. The X-ray dose was 65 K.V. Peak., 5 M.A., 23 centimeters, 31 min. 44 sec. Difference plotted is X-rayed posteriorly—X-rayed entirely.

The results are shown in table 10. In figure 12 the difference in crossing over between the entirely X-rayed and posteriorly X-rayed pupae is

plotted and the probable errors of the differences shown. Clearly at no time during the period under observation, up to ten days after the X-ray treatment, is there a significant difference between the crossover values determined from pupae entirely X-rayed and pupae X-rayed in the posterior half only.

TABLE 11

Experiment 322. Second part. Counts of F_1 from X-rayed and control pupae. The X-ray dose was $30D_{50}$ [50 KV (65 KV Peak) 5 M.A. 23 cm. 31 min. 44 sec.] The pairs remained in the first bottles until 4 days after the treatment, and in the second bottles for 2 days.

BOTTLE	TREATMENT	♀		♂						
		RED LONG	EXCEPTIONS	WHITE		EOSIN		EXCEPTIONS	PERCENT	
				LONG	MIN.	LONG	MIN.		CROSSING OVER	EXCEPTIONS
1	Entire	147	1	51	21	17	41	1	29.2	0.76
	Posterior	122	1	38	16	16	50	6	26.7	4.76
2	Entire	159	1	93	21	22	89	2	19.1	0.88
	Posterior	199	..	69	23	25	75	1	25.0	0.52

In the second part of the experiment female pupae, sisters of those used in the first part of the experiment, were treated as in the first part, 4 entirely and 4 posteriorly, and bred up to six days after treatment. The

TABLE 12

Total of 35+316+320+322

BOTTLE	X-RAY TREATMENT	♀		PERCENT EXCEPTIONS	♂						
		RED LONG	EXCEPTIONS		WHITE		EOSIN		EXCEPTIONS	PERCENT	
					LONG	MIN.	LONG	MIN.		OVER CROSSING	EXCEPTIONS
1	Posterior	500	..	0.00	168	77	62	119	17	32.63	3.84
	Entire	499	1	0.20	178	68	70	148	12	29.74	2.52
	Control	3134	2	0.06	1126	386	380	1147	4	25.21	0.13
2	Posterior	811	1	0.12	338	67	58	332	9	15.72	1.12
	Entire	769	..	0.00	287	58	51	318	9	15.27	1.24
	Control	2960	..	0.00	1040	463	484	1048	5	31.21	0.16
3	Posterior	1301	4	0.31	512	74	82	587	6	12.43	0.48
	Entire	1136	3	0.26	490	59	65	493	6	11.20	0.54
	Control	3173	..	0.00	1197	461	492	1250	3	28.03	0.09
4	Posterior	1326	1	0.08	566	79	119	522	3	15.40	0.23
	Entire	1391	5	0.36	581	120	121	543	2	17.66	0.15
	Control	3406	..	0.00	1256	427	459	1222	2	26.34	0.06

results are shown in table 11 and for the period considered are essentially similar to those for the same period in the first part of the experiment.

COMBINED RESULTS CROSSING OVER IN ALL EXPERIMENTS

The results of X-raying the posterior half and the entire animal in all of the experiments, numbers 315, 316, 320, and 322 have been combined in table 12. This table shows that for crossing over in the eosin to miniature region of the sex chromosome, chromosome I, no significant difference can be observed in any of the four bottles of the control. Although in each of them the crossover values of the X-rayed females are markedly different from those of the control females, those X-rayed entirely and in the posterior only correspond very closely to each other.

TABLE 13

Experiments 315, 316, and 320. Number of exceptions produced according to bottle and treatment. Details of experiments under separate tables.

BOTTLE	TREATMENT	TOTAL NUMBER OF MALES	NUMBER OF EXCEPTIONS	PERCENT OF EXCEPTIONS
1	Anterior	1923	2	.10
	Posterior	49	3	6.12
	Control	2512	4	.16
	Shielded	1770	3	.17
	Entire	105	4	3.81
2	Anterior	1923	3	.16
	Posterior	119	4	3.36
	Control	2528	4	.16
	Shielded	1792	2	.11
	Entire	116	4	3.45
3	Anterior	1922	4	.21
	Posterior	419	6	1.43
	Control	2837	3	.11
	Shielded	1817	2	.11
	Entire	419	4	.95
4	Anterior	1994	..	.00
	Posterior	499	1	.20
	Control	2724	2	.07
	Shielded	1998	1	.05
	Entire	594	1	.17
5	Anterior	2018	2	.10
	Posterior	314	1	.32
	Control	2659	2	.08
	Shielded	1993	4	.20
	Entire	546	1	.18

In the second bottles in which the crossover values of the X-rayed females are approximately 16 percent below that of the control females, the difference between the crossover values of the posteriorly and entirely X-rayed females is only 0.45 percent; in the third bottles the results were somewhat similar, while in the fourth bottles the posteriorly and entirely X-rayed females differ from the control females by approximately 10 percent and among themselves by 2.26 percent.

In the first bottles, however, a difference is to be noticed. Both the entirely and the posteriorly X-rayed show a greater crossover value than the control. These differences, although considerable, are in the case of the entirely X-rayed 1.7 and in the case of the posteriorly X-rayed 3.5 times percent exception difference and therefore may not be significant, especially when taken in connection with the writer's previous findings (MAVOR 1923) where no effect on crossing over was found in eggs laid during the first four days after X-ray treatment.

NON-DISJUNCTION

The numbers of exceptional males occuring in the F_1 of the different categories of X-rayed and control females are given in table 13. This table shows quite clearly that there is a significant increase in the frequency of non-disjunction only in the pupae which were X-rayed entirely or in the posterior half. In the case of the control pupae, the shielded pupae and the pupae which were X-rayed in the anterior half, non-disjunction did not occur more frequently than it does naturally.

SUMMARY

1. The object of the investigation was to determine whether X-ray treatment of Drosophila not involving the reproductive organs would produce an effect on the crossover value and the production of non-disjunction such as produced in previous experiments by X-ray treatment of the entire fly.

2. The technique consisted in shielding the anterior and posterior halves of pupae submitted to X-rays so that one half only received direct radiation. For comparison pupae were also exposed over the entire body, and other pupae were shielded so as to be exposed over the entire body to approximately the same amount of secondary radiation as the shielded halves of the pupae directly radiated over the anterior or posterior.

3. A comparison of the crossover values obtained from pupae X-rayed in the posterior half with those X-rayed in the anterior half and controls not submitted to X-radiation shows that only in the case of the pupae X-

rayed in the posterior half, that is where the ovaries received direct radiation was there a significant difference in the crossover value from that to be expected normally.

4. A comparison of the occurrence of non-disjunction in the differently treated pupae shows that also only in the case of entirely X-rayed pupae and pupae X-rayed in their posterior halves, that is where the ovaries received direct radiation, can a significant increase in the production of non-disjunction be recognized.

5. In some of these earlier experiments a difference in the time of incidence of the reduction of the crossover value between pupae entirely X-rayed and X-rayed in the posterior half only was noticed. This was not found in later experiments and may or may not be signficant.

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THE GENETICS OF PLATYPOECILUS. II. THE LINKAGE OF TWO SEX-LINKED CHARACTERS[1]

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Of recent years investigators in the field of genetics have been giving increasing attention to fishes, especially those kinds which can be reared in aquaria. Among the latter the smaller killifishes of the order Cyprinodontes have proved well suited to inheritance studies. Certain species of this order present a variety of bright colors and a wide range of melanic patterns, as well as marked size differences, all of which characters attract the interest of the student of heredity. The small size of most of these fishes makes it possible to rear comparatively large numbers in a limited space. As an added advantage, they have a relatively short life cycle. To be sure there are difficulties attending their culture, such as exacting temperature requirements, problems in feeding and handling, as well as in

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disease control and in general aquarium management; but these can be overcome.

In an earlier paper, GORDON (1927) has discussed the taxonomy and life history of *Platypoecilus maculatus* and presented the results of a study of the inheritance of two types of melanophores: a micro-melanophore responsible for the character called *stipple* (S_t) and a macro-melanophore giving the character called *spot* (S_p). The Stippled race was shown to differ from the Non-stippled one by a single dominant autosomal gene, while the Spotted race differed from the Non-spotted by a dominant sex-linked gene. GORDON also confirmed the report of BELLAMY (1922) that in *Platypoecilus maculatus* sex determination is of the W Z type.

The present report deals with the factor for *red* body-color and its relation to the factor for *spots*. *Red* is shown to be sex-linked, thus confirming BELLAMY'S findings. Data are presented which seem best explained on the basis of linkage between *spots* and *red*, with occasional crossing over between them in the W and Z chromosomes[4] of the female.

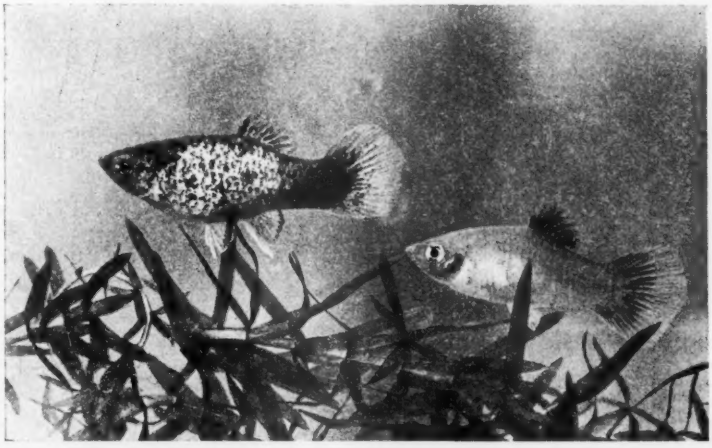

FIGURE 1.—*Platypoecilus maculatus*—about natural size. Left: Red, *spotted* female. Right: Gold male (*non-red, non-spotted*). The reflection of the light on the Red fish is due to an iridescence not genetically associated with the *red* character.

[4] In general the writers favor the more recent practice of using XY to represent the heterogametic sex and XX the homogametic in both the Abraxas and Drosophila types of sex linkage. In this case, however, there seem to be certain advantages in the use of the earlier forms: WZ and ZZ.

MATERIALS

In these studies two strains of *Platypoecilus maculatus* were used: the Red or Rubra type and the Gold. Both of these have been described and pictured in color in an earlier paper (GORDON 1927).

The male of the Red variety is Brazil Red (RIDGWAY, plate 1) at maturity. In this sex the young fishes show no red color. The red pigmentation usually begins to appear at the stage when the anal fin has almost completed its transformation into the intromittent organ. Occasionally the assumption of red color may be delayed well beyond that time. In one or two cases males failed to take on color even though they were genetically Red. These fishes looked abnormal in other respects. An examination showed that their gonads were either abnormal or lacking. This seems to indicate a relation between normal male gonads and the capacity to develop red color. The delay in development of red by the male makes it necessary to keep the males for a comparatively long time in order to give all those which are genetically Red an opportunity to develop red color.

The female of the Red variety is a dull olive brown color, often becoming "Cinnamon Rufous" (RIDGWAY, plate 14). From BELLAMY's description (1924) of his Red strain it would appear that the females were colored red like the males, but that the color developed much more strongly in the males. One of the writers has seen in the collections of aquarium fish fanciers, females of *Platypoecilus maculatus* which were distinctly reddish. It would seem possible that the same primary factor for red pigmentation is involved in both of these color phases, since both show sex linkage. Its expression in the mature fish may be influenced by environmental conditions, by sex hormones, or by a modifying gene or genes either in the autosomes or in the W or Z chromosomes.

From these descriptions it will appear that in the races used there is a sexual bicolorism, with *red* sex-limited in the males. The *red*-type females can usually be distinguished from those of the Gold race, with which they were crossed, by their brownish coloration. The designation *red-type female* is used here to refer to these brownish or olive brown females, not to fish which show distinct red color.

The Gold variety of *Platypoecilus maculatus* was used as the other parent in these crosses, chiefly because the body exhibits no red color and no spots. GORDON (1927) gives the body color as deep Colonial Buff (RIDGWAY, plate 30), with the dorsal fin of the male Scarlet and that of the female LaFrance pink (RIDGWAY, plate 1). The males and females of the Gold race are quite distinct from those of the Red and no difficulty

was experienced in classifying the progeny of the various crosses. Occasionally a few erythrophores may be seen in the Gold variety, forming a reddish saddle on the back, but these are confined to the region just below the dorsal fin and do not change the general yellowish coloration of the animal.

PARENT RACES TRUE-BREEDING

Preliminary studies of the Red variety and periodic examinations of the stock of it, have shown that is is a true-breeding race. Of more than 75 Red fish which have been reared, all the males have been red and the females have been the characteristic brown. These females in turn have given only red male and female offspring like themselves. In a similar test the Gold has proved to be a true-breeding race. Approximately 100 young of this variety have been raised.

CROSSES OF GOLD AND RED

In the following crosses between the Red and Gold races the numbers of fish reared to adult size are not always as large as might be desired. This was due chiefly to limitations imposed by the cultural requirements and to troubles with gill parasites. In order to rear these fishes successfully it was necessary to glass in a part of a large laboratory and to equip this smaller room with thermostatically controlled heating units and with artificial lighting. This limited the space available for aquaria. Then too, the delayed development of red color in the males made it necessary to keep them for a long time and to use for them aquaria which might have been occupied by other schools. In spite of these handicaps, however, the numbers are sufficiently large to be indicative of certain types of behavior.

TABLE 1

Cross of Gold ♀ (non-spotted, non-red) × Red ♂ (spotted, red).

$Z\ r\ s_p\ W\ r\ s_p \times Z\ R\ S_p\ Z\ R\ S_p$

GENERATION	PEDIGREES*	RED				NON-RED			
		Spotted		Non-spotted		Spotted		Non-spotted	
		♀	♂	♀	♂	♀	♂	♀	♂
F_1	14, 15	18 (20)†	22 (20)	0	0	0	0	1 (0)	0
F_2	28, 29, 30, 38	33 (28.5)	47 (57)	0	0	0	0	34 (28.5)	0

* Separate records have been kept for each mating, but in most cases the data have been combined where they seemed clearly to show the same behavior.

† Calculated frequencies are shown in parentheses.

The cross Gold ♀ × Red ♂ gave an F_1 progeny of 40 red, spotted animals and 1 non-spotted, non-red female. In the first class there were about equal numbers of males and females. The single exceptional female died before it could be tested further. It is possible to account for this individual by assuming that an egg of the type **Z** *r* s_p was fertilized by a sperm which did not contain any Z chromosome. (W and Z in Roman bold type refer to chromosomes and are to be distinguished from the following factor symbols which are in italics). This second type of gamete could have been produced by non-disjunction in the male.

In the second generation, definite evidence of sex linkage is found. The data are in fairly good agreement with the calculated 1:2:1 ratio (P = .1769) and only females appear in the non-red, non-spotted class. These F_2 results suggest either that *red* and *spots* are caused by the same gene[5] or that two closely linked genes are involved and that there is little crossing-over between them.

TABLE 2

Cross of Red ♀ (red, spotted)×Gold ♂ (non-red, non-spotted).
Z *R* S_p **W** *r* s_p × **Z** *r* s_p **Z** *r* s_p.

GENERATION	PEDIGREES	RED				NON-RED			
		Spotted		Non-spotted		Spotted		Non-spotted	
		♀	♂	♀	♂	♀	♂	♀	♂
F_1	16, 17, 36, 37	0	47 (49)	0	0	0	0	51 (49)	0
F_2	39, 40, 53, 54	14 (12)	13 (12)	0	0	0	0	13 (12)	9 (12)

The F_1 and F_2 results of the cross Red ♀ × Gold ♂ (table 2) bear out the assumption that sex-linked genes are operating. The F_1 shows criss-cross inheritance of the WZ type. While the F_2 frequencies are not large, they agree rather closely with the results calculated for 49 individuals. The 1:1 distribution within each sex is typical of the F_2 in a case of sex linkage where the F_1 shows criss-cross inheritance.

Two backcrosses of the F_1 females of table 1 (Gold ♀ × Red ♂) to pure recessive (Gold) males, yielded 85 young.[6] These were distributed in a manner similar to the F_1 shown in table 2. In both of these cases, the red,

[5] It is not held, of course, that one gene is solely responsible for each of these characters, but rather that the *difference* between *red* and *non-red*, or *spotted* and *non-spotted*, is due largely to one gene. The terms "gene for *red*" and "gene for *spots*" are used for convenience.

[6] The complete data may be found in table 3 on the page following.

spotted females (P_1 and F_1, respectively) seem to be heterozygous for the dominant sex-linked genes for *red* and *spots*. Farther on, genetical evidence will be presented which indicates that the W chromosome is present in the female of this species, but that it usually carries only recessive genes. None of the above results admit of an explanation on the basis of autosomal genes which are in the heterozygous condition in the P_1 female.

TABLE 3

Backcross of the red, spotted F_1 ♀ of the cross Gold ♀ × Red ♂ (table 1) to Gold ♂ (non-red, non-spotted).

$$Z\ R\ S_p\ W\ r\ s_p \times Z\ r\ s_p\ Z\ r\ s_p$$

GENERATION	PEDIGREES	RED				NON-RED			
		Spotted		Non-spotted		Spotted		Non-spotted	
		♀	♂	♀	♂	♀	♂	♀	♂
	33	0	12	0	0	0	0	10	0
F_2	52	1*	29	0	0	0	0	33	0
Total		1	41	0	0	0	0	43	0

* This exceptional individual (52–20) has been tested further. See tables 6 and 8.

These backcrosses constitute good proof of the sex linkage of both *red* and *spots*. The F_1 females, although they do not show red color, presumably have received genes for *red* and *spots* from their male parents, and they are considered to be of the genotype $Z\ R\ S_p W\ r\ s_p$. When these females are crossed to Gold (non-red, non-spotted) males, the appearance of red, spotted male offspring and non-red, non-spotted females shows that the F_1 females were carrying the genes for *red* and *non-red*, *spotted* and *non-spotted* in the chromosomes which differentiate between maleness and femaleness.

One exceptional female (pedigree 52-20) appeared among the progeny: a red, spotted animal. She was mated to two different males and the results of those matings are given in tables 6 and 8. This red, spotted female seems best explained on the basis of crossing over between the W and Z chromosomes of the F_1 female (table 3). Evidence to support this belief will be presented farther on.

The backcross of the F_1 non-spotted, non-red females resulting from the cross Red ♀ × Gold ♂ (table 2) to Gold males, gave only non-spotted, non-red fishes in F_2 with males and females in approximately equal numbers.[7] Such results are to be expected if the P_1 red, spotted female (table 2) fails to transmit either *red* or *spots* to her female offspring.

[7] See table 4 on page following.

TABLE 4

Backcross of the non-red, non-spotted F_1 ♀ of the cross Red ♀ × Gold ♂ (table 2) to Gold ♂ (non-red, non-spotted).

$Z\ r\ s_p\ W\ r\ s_p \times Z\ r\ s_p\ Z\ r\ s_p$.

GENERATION	PEDIGREES	RED				NON-RED			
		Spotted		Non-spotted		Spotted		Non-spotted	
		♀	♂	♀	♂	♀	♂	♀	♂
F_2	49, 50	0	0	0	0	0	0	10	15
	71, 72	0	0	0	0	0	0	35	47
	99, 100	0	0	0	0	0	0	25	22
Total		0	0	0	0	0	0	70	84

A cross of the red, spotted F_1 male of table 1, to a red, spotted, stock female, gave red, spotted fish and non-red, non-spotted ones. If the genes for *red* and *spots* are sex-linked, all of the F_2 males should have been spotted and red, and the females should have been of two kinds: spotted and red, and non-spotted and non-red. For some unknown reason, most of these fish died before becoming differentiated as to sex. Two red, spotted animals turned out to be males and a single *non-spotted fish also developed into a male*. Later on this non-spotted animal took on full *red color*.[8] Both the development of red color and the assumption of maleness by this non-spotted fish were contrary to theoretical expectations. This exceptional type will be referred to later on under the number 32–11.

TABLE 5

Cross of the red, spotted F_1 ♂ of the cross Gold ♀ × Red ♂ (table 1) to a red, spotted stock female.

$Z\ R\ S_p\ W\ r\ s_p \times Z\ R\ S_p\ Z\ r\ s_p$.

PEDIGREE	RED		NON-RED	
	Spotted	Non-spotted	Spotted	Non-spotted
32	14	1	0	2

THE EXCEPTIONAL FEMALE (NO. 52–20) OF TABLE 3

In the backcross of an F_1 red, spotted female (Cross = Gold ♀ × Red ♂) with a Gold male, a single female appeared which was red and spotted. Attention has been called to this above. Several possible explanations of this exception suggest themselves: (1) that it was due to non-disjunction, (2) that gene mutation caused it, (3) that it resulted from a case of sex

[8] See table 5.

reversal, and (4) that it was due to crossing over between W and Z in the F_1 female.

The parents of the cross reported in table 3 may be represented as in figure 2.

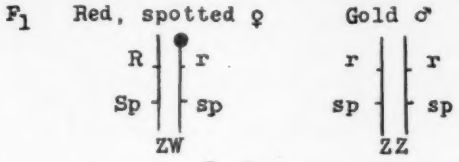

FIG. 2.

1. *Non-disjunction*

Case (a)

If the $Z\,R\,S_p$ gamete of the female shown above had been fertilized by a *zero* type of sperm, (the result of non-disjunction in the male), it would have given a fish of the type $Z\,R\,S_p\,O$. This might very well have been female in sex, judging from the case in *Drosophila melanogaster*, where the XO type is male and where the mode of sex determination is the reverse of that in Platypoecilus. The fact that an XO male in Drosophila is sterile does not necessarily mean that a ZO female in Platypoecilus would also be sterile.

Case (b)

If non-disjunction had occurred in the W and Z chromosomes of the F_1 female and these had gone together into the egg, a subsequent fertilization with the $Z\,r\,s_p$ sperm of the Gold male would have given a fish of the constitution $Z\,R\,S_p\,W\,r\,s_p\,Z\,r\,s_p$. The corresponding type in Drosophila, namely XXY, is female in sex. This ZZW fish might be expected to be a male on the same basis, because of the presence of two Z chromosomes. This type of reasoning does not prove, however, that non-disjunction in the F_1 female would produce F_2 exceptional males instead of females. A fish of the constitution ZZW, should exhibit secondary non-disjunction and subsequent tests should throw light upon this possibility.

2. *Mutation*

The exceptional red, spotted female of table 3 could have been produced by mutation, but from what is known of the frequency of gene mutations this possibility seems remote. It would necessitate a coincidental mutation in two genes, r and s_p, either in the W chromosome of the F_1 female or in the Z chromosome of the Gold male, unless R–S_p and r–s_p are each one gene. The writers believe that R and S_p are better accounted for by assuming two distinct genes.

3. *Sex reversal*

There is also a possibility that this exceptional female was genetically a male, but that it later became transformed into a female by a process of sex reversal. A number of cases of this kind have been reported. ESSENBERG (1923 and 1926) described cases of sex reversal in a closely related fish *Xiphophorus helleri*. CREW (1927) refers to a number of reported cases of hermaphroditism in fishes, which he is inclined to believe may represent transition stages in the process of sex reversal. Enough work has been done, at least, to show that the phenomenon of sex reversal is probably not an uncommon one among fishes, and particularly among those forms related to the genus Platypoecilus.

CREW (1927) says regarding individuals which have been transformed as to sex.

> "If in a group there is a sex-chromosome, sex-determining mechanism (for example, XY:XX) and if this mechanism can be over-ridden, then in that group it can be expected that there will be individuals genotypically of one sex, phenotypically of the other, and that these when mated to individuals in which sexual genotype and phenotype are in agreement will produce offspring among which there will be a preponderance of the sex to which the transformed parents genotypically belonged (that is, a transformed female functioning as a male will yield a preponderance of females; a transformed male functioning as a female will yield a preponderance of males)." (p. 438).

If the exceptional female of table 3 was genotypically male, she must have been of the constitution **Z** *R* S_p **Z** *r* s_p. Such a female when crossed with a normal (ZZ) would give only male offspring, barring other cases of sex reversal. In as much as this female gave 8 females among her offspring in one cross (table 6) and 15 females in the progeny of another cross (table 8), it seems very unlikely that she could have been an F_1 male in which sex reversal had taken place.

4. *Crossing over*

Crossing over between the W and Z chromosomes in the F_1 female of table 3, would have given a red, spotted female among the progeny of the backcross. The F_1 female was assumed to be of the constitution **Z** *R* S_p **W** *r* s_p. If the genes for *red* and *spots* were transferred to the W chromosome by crossing over in this F_1 female, and the egg which received the W chromosome was fertilized by a sperm bearing **Z** *r* s_p, the resulting fish would be a female of the constitution **Z** *r* s_p **W** *R* S_p. Subsequent tests of this exceptional female (tables 6 and 8) lend strong support to the belief that she was actually of this constitution.

By the time this female no. 52–20 was recognized as an exceptional

type, she had already mated with a red, spotted male of the same F_1 family. The results of that mating are given in table 6.

TABLE 6

Cross of the exceptional red, spotted female of table 3, to a male of the same family (No. 52).

PEDIGREES	RED				NON-RED			
	Spotted		Non-spotted		Spotted		Non-spotted	
	♀	♂	♀	♂	♀	♂	♀	♂
52-20×52-11	8	8	0	0	0	0	0	6

The males of pedigree 52 were red and spotted (see table 3) and presumably of the genotype $\mathbf{Z}\ R\ S_p\ \mathbf{Z}\ r\ s_p$. If the exceptional female (52-20) had been like the ordinary red, spotted females and of the constitution $\mathbf{Z}\ R\ S_p\ \mathbf{W}\ r\ s_p$, a cross with one of the F_1 males should have given red, spotted males and females, and non-red, non-spotted females only (no crossovers considered here). On the other hand if female 52-20 had been produced by crossing over between chromosomes W and Z in such a way as to place genes *R* and S_p on the W chromosome, a cross with a male of family 52 would have given results as follows:-

$$\mathbf{Z}\ r\ s_p\ \mathbf{W}\ R\ S_p \times \mathbf{Z}\ R\ S_p\ \mathbf{Z}\ r\ s_p.$$

$$F_2$$

$\mathbf{Z}\ r\ s_p\ \mathbf{Z}\ R\ S_p$—red, spotted ♂.
$\mathbf{Z}\ r\ s_p\ \mathbf{Z}\ r\ s_p$—non-red, non-spotted ♂.
$\mathbf{Z}\ R\ S_p\ \mathbf{W}\ R\ S_p$—red, spotted ♀.
$\mathbf{Z}\ r\ s_p\ \mathbf{W}\ R\ S_p$—red, spotted ♀.

The results shown in table 6 check with the assumption that female no. 52-20 was of the genotype $\mathbf{Z}\ r\ s_p\ \mathbf{W}\ R\ S_p$. They also rule out the possibility that such a female was produced by non-disjunction of type 1a (above), that is, that her genotypic constitution was $\mathbf{Z}\ R\ S_p\ 0$. Non-disjunction of type 1b would give a fish of the genotype $\mathbf{Z}\ R\ S_p\ \mathbf{W}\ r\ s_p\ \mathbf{Z}\ r\ s_p$, probably a male. If it were possible for such a fish to be a female a cross with a male of family 52 would give a number of non-spotted, non-red females in the next generation.

It would seem, therefore, that the results in table 6, effectively eliminate suggestions 1a, 1b, and 3, regarding the genotype of the exceptional female, 52-20. This table does, however, clearly support assumption 4, namely that the exceptional female resulted from a crossing over between the W

and Z chromosomes in her maternal parent. Two other tests furnish additional evidence of a similar nature.

The red, spotted females (table 6) arising from the cross ♀ 52-20 × ♂ 52–11, were mated to Gold (double recessive) males. Table 7 gives the results of these matings. The offspring are classified only for *spots*, since none of them are as yet old enough to develop red color. Judging from the high degree of association between *red* and *spots* in the preceding crosses, most if not all of the spotted young will probably be red, and the non-spotted will be non-red.

TABLE 7

Test of the red, spotted females of table 6 by crossing to a (r s_p) Gold male.

PEDIGREE OF ♀ TESTED*	SPOTTED YOUNG	NON-SPOTTED YOUNG	PROBABLE GENOPTYE OF PARENT
121–1	13	0	W Z S_p S_p
121–2	3	4	S_p s_p
121–3	3	4	S_p s_p
121–5	41	0	S_p S_p
121–6	85	0	S_p S_p
121–7	69	0	S_p S_p

*Females 121–4 and 121–8 died before producing any young fishes.

If the exceptional female 52–20 was of the constitution **Z** r s_p **W** R S_p, her red, spotted female offspring shown in table 6 should have been of two kinds: **Z** R S_p **W** R S_p and **Z** r s_p **W** R S_p. The first of these when mated to a Gold male should give only red, spotted young, males and females. The second type should give non-red, non-spotted male offspring and red, spotted female offspring. Females no. 121–1, 121–5, 121–6 and 121–7 are undoubtedly of the first type. They represent an unusual genetic condition: homozygosity of sex-linked genes in the heterogametic sex. Females 121–2 and 121–3 are clearly of the second class mentioned above. Both kinds of females are of further interest because they seem to carry dominant, sex-linked genes in the W chromosome and they should (barring subsequent crossing over) transmit these genes to their daughters and thence to their granddaughters and so on, in a manner similar to the one-sided inheritance of *maculatus* in *Lebistes reticulatus* (SCHMIDT 1920 and WINGE

1922) and *bobbed* in *Drosophila melanogaster* (STERN 1926). The present case, however, would be one of one-sided inheritance in the female line.

After the exceptional red, spotted female (52–20) of table 3 had given birth to her young by the mating with the F_1 red, spotted male (see table 6), she was crossed with a Gold male. The results of that cross are given in table 8.

TABLE 8

Cross of the exceptional red, spotted female (table 3) to a Gold (r, s_p) male.

PEDIGREES	RED SPOTTED		NON-RED NON-SPOTTED	
	20		28	
	♀	♂	♀	♂
52–20×99–11	15	1	0	17

If female 52–20 was genetically **Z** $r\ s_p$ **W** $R\ S_p$, a cross with a Gold male should have given red, spotted daughters (like the mother) and non-red, non-spotted sons (like the father). Fifteen spotted females and seventeen non-spotted male offspring support this conclusion regarding the genotype of female 52–20. The single, red, spotted male may be the result of crossing over between the W and Z chromosomes of this exceptional female to place the genes for *spots* and *red* back on the Z chromosome. At least it is possible to account for it on this basis.

These tests of female 52–20 seem clearly to support the original assumption that she was of the constitution **Z** $r\ s_p$ **W** $R\ S_p$. Such a fish could only have been produced by crossing over between the W and Z chromosomes of the F_1 female.

EXCEPTION NUMBER 2.

In the discussion of the results given in table 5, reference is made to a non-spotted, *red* male which appeared in the offspring of an F_1 male heterozygous for *red* and *spots*, crossed to a stock red, spotted female. The parents in this cross may be represented as follows: **Z** $R\ S_p$ **W** $r\ s_p \times$ **Z** $R\ S_p$ **Z** $r\ s_p$. The exceptional red, non-spotted male (32–11) is thought to have been produced by the union of a sperm of the constitution **Z** $r\ s_p$ with an egg carrying **Z** $R\ s_p$. The odds seem strongly against its having been produced in any other way. The female gamete in this case could have been produced in either one of two ways: by crossing over between the W and Z chromosomes of the female parent, with the break coming between the genes R and S_p; or by mutation of S_p to s_p on the Z chromosome of the female. Either process would give the same end result, and it is hard to see how

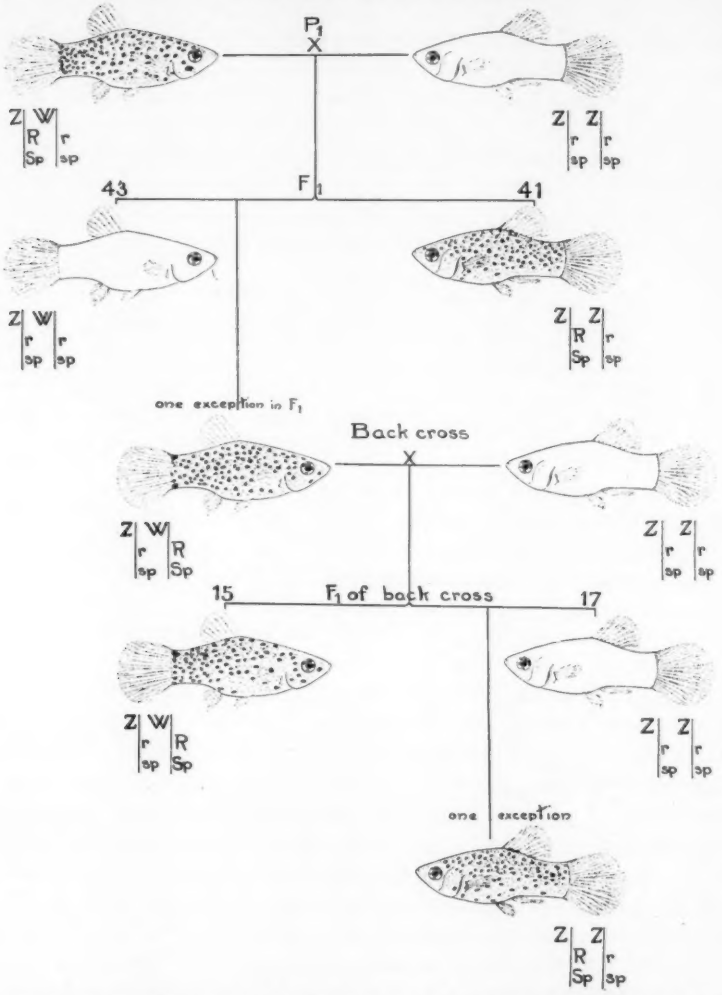

FIGURE 3.—This illustrates the breeding behavior of a normal, red, spotted female (pedigree number 15–2) when crossed with a double recessive, gold, male (pedigree number 20–11). It shows definite criss-cross inheritance of two sex-linked genes R and S_p in F_1. A single exception occurred: a red, spotted female (pedigree number 52–20). This individual's appearance is accounted for by crossing over in the sex chromosomes of the P_1 female, resulting in a transfer of the genes R and S_p from the Z to the W.

The exceptional red, spotted F_1 female is backcrossed to a double recessive male (pedigree number 99–11). The results show a different sort of inheritance: the dominant genes R and S_p are transmitted from mother to daughter and their recessive allelomorphs r and s_p from father to son. Again an exception appeared: a red, spotted male. It is accounted for by crossing over in the sex chromosomes of the F_1 exceptional female, resulting in the transfer of the dominant genes R and S_p from the W back to the Z.

Note: Females are to the left of the cross (X), males are to the right.

one could tell with any degree of certainty which one did occur. However, in view of the fact that crossing over seems to have occurred between the W and Z chromosomes of a female of Platypoecilus in the case of female 52–20, the writers are disposed to consider this male (32–11) as very probably the result of a similar process.

A cross of this exceptional male (32–11) to a Gold female gave results which showed that the male was heterozygous for *red* and was genetically *non-spotted*. The results of this cross are shown in table 9.

TABLE 9

Mating of Gold ♀ (stock) × the non-spotted, red ♂ of table 5.

PEDIGREES	RED NON-SPOTTED		NON-RED NON-SPOTTED	
	♀	♂	♀	♂
42–2×32–11 ♀ ♂	5*	13	20	12

* In this cross the females were hard to classify for red and non-red.

DISCUSSION

The data presented above show that the characters *red* and *spots* are sex-linked. From a theoretical standpoint these characters might be considered as due to either one of two conditions: first, a single gene influencing both *red* and *spots*, with the various combinations of these characters comprising a multiple allelomorphic series; secondly, a distinct gene for each character with a rather close linkage of the two. The first case is shown not to apply here by the appearance of new combinations of the characters *red* and *spots*. A linkage of the genes for *red* and *spots* is best shown by a close association of these characters in heredity, with occasional breaks between them, and the data given above answer this requirement.

AIDA (1921) seems to have been the first to report crossing over between unlike sex chromosomes (X and Y). In his studies of inheritance in *Aplocheilus latipes*, another small fish of the order Cyprinodontes, he obtained evidence that a gene for red body-color (*R*) was carried both by the X and the Y chromosomes. This gene was found to crossover from X to Y, or Y to X in heterozygous animals. Several types exceptional with reference to color and sex seem best explained on that basis.

WINGE (1923), working with *Lebistes reticulatus*, another Cyprinodont fish, reported crossing over of a factor for elongated caudal fin (elongatus: *e*) from the X to the Y chromosome, and by further crossing over, a return

of *e* to the X chromosome. More recently (WINGE 1927) has described five genes (luteus, elongatus, minutus, vitellinus, and cinnamomeus) which have crossed over in different ways from X to Y, Y to X, or X to X, with crossing over occurring in both sexes.

Crossing over between the like chromosomes of the homogametic sex has long been known to occur in Drosophila and in fowls. The work with fowls has shown that crossing over can take place between the Z chromosomes of the male. GOODALE (1917) reported crossing over between the gene for barred pattern and that for silvering of the hackle, when F_1 males were backcrossed to recessive females. HALDANE (1921) confirmed this and gave a crossover percentage for *B* and *S* of 34.6 ± 3.6. SEREBROVSKY (1922) added a third sex-linked gene, "suke", for retarded development of feathering in chicks, and showed that this gene is linked with the factors for bar and silvering (which he called "trage" and "tuge"). Crossing over between these occurred in the male, but not in the female. This is to be expected, of course, if in the female there is no mate for the Z chromosome. (HANCE (1926) reports an unpaired sex chromosome in the female of fowls). AGAR (1924) and HALDANE and CREW (1925) have confirmed the findings of SEREBROVSKY, the latter workers reporting an increase in percentage of crossing over as the males got older.

The present work with Platypoecilus seems to include the first case of crossing over between the sex-chromosomes of an heterogametic female (that is, between W and Z). Crossing over between the X and Y (or W and Z) chromosomes in the heterogametic sex is of considerable theoretical interest; first, from the standpoint of its effect upon the sex-determining mechanism, and secondly, as it affects the study of sex-linkage.

The more recent theory of BRIDGES (1922 and 1925) regarding the genetical nature of sex appears to have been well received by geneticists. (This theory has proved helpful to an understanding of the possible genetic nature of all characters). Evidence that the autosomes play a part in the determination of sex is convincing. The consequent assumption seems warranted that there are many genes for maleness and femaleness scattered through the whole chromosome complex, and that the sex of the individual is determined by the algebraic sum, so to speak, of all these genes. According to this theory the sex chromosomes still control the situation. When two of them are present, the overbalance of femaleness in the two X's is sufficiently greater than the overbalance of maleness in the autosomes to make the individual a normal female. When only one X is present, the balance is decidedly in favor of maleness. As the writers understand this theory, the members of any pair of autosomes are geneti-

cal equivalents with respect to these genes for sex, and crossing over between them should cause no change in the sex of the individuals receiving the chromosomes which have interchanged parts. In the same way the two X's are genetical equivalents with respect to the sex genes and crossing-over between them should produce no new types of chromosomes as far as the genes for sex are concerned. The X and Y chromosomes, however, are genetically different with respect to the sex genes. Crossing over between these chromosomes should result in a wholly new type of internal balance and consequently an appreciable number of intersexes.

Neither AIDA nor WINGE mentions the occurrence of intersexes in the course of his experiments and none were detected in the present work with Platypoecilus. This raises a question as to the nature of the sex-determining mechanism in this group of fishes.

AIDA speaks of the crossing over of the *R* gene from the Y to the X chromosome, but does not subsequently explain what would be the role of such a chromosome, part X and part Y. WINGE simplifies matters by assuming that the X chromosome in Lebistes carries a gene for femaleness and the Y chromosome, a gene for maleness. These are allelomorphic and hence cannot both lie in the same chromosome. In the various crossings over, that chromosome which receives the gene for maleness becomes the Y chromosome. This implies a belief that the genetic difference between maleness and femaleness in Lebistes lies chiefly in one pair of genes. (Later, in reply to a criticism, WINGE (1927) modifies his earlier conception as follows: "In Lebistes, also, the autosomes contain dispositions towards both male and female sex characters; the presence or absence of the *Y* gene is only that which *normally* regulates the sex determination." The statement that the autosomes influence sex is unsupported by published evidence.)

In Platypoecilus the evidence from sex linkage shows that there is one pair of chromosomes which differentiates between maleness and femaleness. There is crossing over between the W and Z chromosomes of the female and no intersexes result. Hence the conclusion seems warranted that the regions in which crossing over occurs are not regions of *difference* between W and Z, at least with respect to any very potent sex factors. (MORGAN (1926) says: "This means no more than that one part only of the sex-chromosomes has the function of an X– or Y–chromosome. . . . .") If there are sex genes in these regions, they must be the same in both the W and Z chromosomes. The portions of W and Z which are similar (for sex genes) are thus essentially autosomal in nature, as MORGAN (1926) has pointed out. The writers are assuming, tentatively, that the

difference between W and Z lies in a very few genes which tend to remain associated in heredity, either because of close proximity or some influence which prevents the occurrence of breaks between them. As in the case of WINGE'S assumption, that chromosome which gets one set of genes is genetically the W chromosome and its mate is the Z.

While there is no evidence that the autosomes of Platypoecilus play a part in sex determination, the above assumptions can be brought into harmony with such a belief if it is so desired. The autosomes can be assumed to carry an overbalance of genes for femaleness, but not in great amount. It is necessary to assume that the genes in the area which distinguishes Z from W are very potent and that the balance in Z is decidedly toward maleness. Values will have to be adjusted to allow for the possible appearance of intersexes between the true conditions of maleness and femaleness.

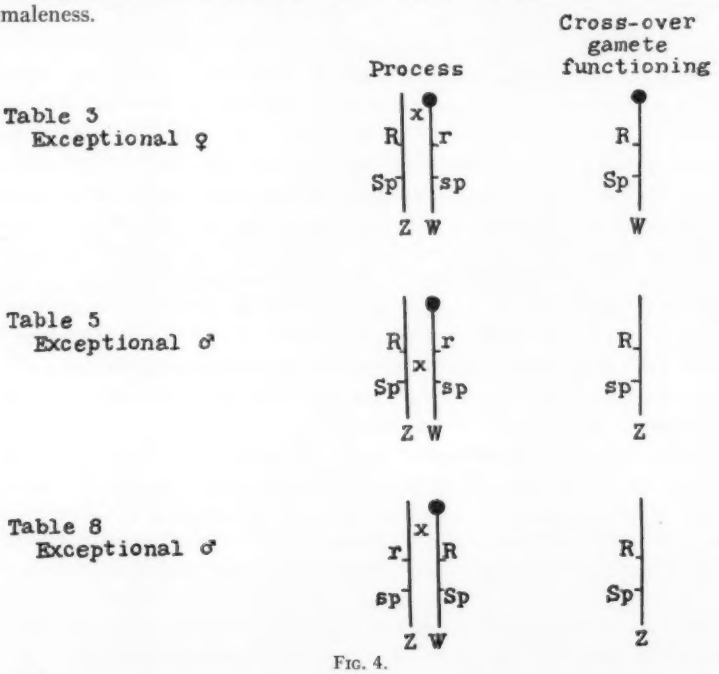

FIG. 4.

The diagrams in figure 4 show where crossing over is believed to have taken place in the course of this work with Platypoecilus. The darker area at the end of one chromosome distinguishes the W chromosome from the Z. It can be thought of as that portion in which Z and W are unlike

with respect to important sex genes, although there is no evidence that such a region is located at the end of the chromosome.

Crossing over between the W and Z chromosomes of the female in Platypoecilus may give peculiar types of offspring in which the sex linkage relations are confusing. A normal red, spotted female of *Platypoecilus maculatus* is presumably of the constitution **Z** $R\ S_p$ **W** $r\ s_p$. Such a female when crossed with a non-red, non-spotted male gives red, spotted sons and non-red, non-spotted daughters in F_1 and both types equally represented by males and females in F_2. If however, the genes R and S_p cross over together from the Z to the W chromosome, it is possible to get by subsequent crosses two types of females: **Z** $R\ S_p$ **W** $R\ S_p$, that is a female homozygous for dominant sex-linked genes, and **Z** $r\ s_p$ **W** $R\ S_p$, which is just the reverse of a normal female of the red variety. The first type of female when crossed with a Gold male ($r\ s_p$) would give all red, spotted offspring in F_1, and in F_2 (not considering crossovers), 3 red, spotted fish to 1 non-red, non-spotted one, the latter occurring only in the males. Such behavior is characteristic of the XY type of sex linkage. These results are similar to those obtained in the F_2 of the cross red-eyed female Drosophila X white-eyed male, where the F_2 recessives (whites) are all males. Here then is the unusual situation of an organism which normally exhibits the WZ type of sex determination, but which in some individuals can show the XY type of sex linkage. Fortunately the reciprocal of the cross listed above gives the WZ type of sex linkage.

AIDA obtained results similar to these in his studies of the sex-linked gene for red body color in Aplocheilus. His cross of a white female ($X\,r\ X\,r$) with a red male ($X\,R\ Y\,R$) gave in F_2 (Experiment 7) 3 reds to 1 white, with only females in the white class. This behavior, however, is characteristic of the WZ type of sex determination. AIDA speaks of these as "peculiar results" but does not point out their importance from the standpoint of studies on sex linkage.

From these facts it would seem to be permissible to make the following generalizations. (1) Where, of a pair of sex-determining chromosomes (XY or WZ), the heterochromosome (Y or W) does not bear any genes which are dominant over recessives on the X (or Z) chromosomes, the genetical evidence from sex linkage furnishes a reliable index of the mode of sex determinaton. (2) Where both sex chromosomes in the heterogametic sex may carry dominant, sex-linked genes, an individual of the constitution XY or WZ can be homozygous dominant and tests for sex linkage are likely to indicate a sex-determining mechanism just the reverse of that which actually operates.

CHROMOSOMES

DOCTOR BARBARA MCCLINTOCK of the Department of Botany, CORNELL UNIVERSITY, has kindly made some preliminary studies of the chromosomes of the male of *Platypoecilus maculatus*, using both sections and Belling smears. The chromosomes in these preparations are numerous and small in size, so that it is hard to make an accurate count of their number without a much more intensive study of this material.

SUMMARY[6]

1. Data are presented on the results of crosses between the Red, *spotted*, and the Gold races of *Platypoecilus maculatus*.

2. The characters *red* and *spots* in the Red race are apparently due to distinct dominant genes both of which are carried on the sex chromosome, the W chromosome usually bearing only recessive genes.

3. Occasionally crossing over occurs between the W and Z chromosomes of the female to interchange these dominant genes with their recessive allelomorphs. It has not yet been determined whether crossing over takes place between the two Z chromosomes of the male.

4. The transference of dominant genes from the Z to the W chromosome gives rise to females which exhibit one-sided feminine inheritance.

5. In some of these experiments females were obtained which, although they were of the heterogametic sex, nevertheless carried dominant sex-linked genes in the homozygous condition.

6. The nature of the sex-determining mechanism is considered.

POSTSCRIPT

Soon after the above manuscript was sent to the editor of Genetics, A. W. BELLAMY's second paper on Platypoecilus arrived at this laboratory (Genetics 13: 226–232, 1928). The problems and interpretations contained in this paper are essentially the same as those which have previously been communicated by him. They do not, therefore, require any additional comment at this time.

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[6] See Science **67:** 470, 1928.

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A CYTOLOGICAL AND GENETICAL STUDY OF TRIPLOID MAIZE

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INTRODUCTION

During the course of investigation on the chromosome number and behavior of different genetic strains of maize a triploid individual appeared in an otherwise diploid culture. As has been reported in a preliminary note (RANDOLPH and MCCLINTOCK 1926), this individual was notably more vigorous than its diploid sibs. Since the triploid possessed certain known genetic characters it was desired to follow the genetics along with the cytology in the descendants of this individual.

In the preliminary note it was suggested that the triploid probably arose through the fusion of a diploid and a haploid gamete. Since the publication of this note much evidence has accumulated which would support this probability (see pp. 209 and 209; see also figures 17 and 27).

The literature on Zea chromosomes is rapidly becoming more extensive. Since Miss FISK (1927) has reviewed most of the existing literature on the subject (KUWADA 1911, 1915, 1919, 1925; LONGLEY 1924, 1925; FISK 1925; KIESSELBACH and PETERSEN 1925) little more need be said with regard to

previous investigations on Zea. To this list we might add the short paper of REEVES 1925, in which it is stated that of 8 varieties of maize examined, 7 showed 10 pairs of chromosomes during meiosis; the eighth, a Black Mexican variety, on the other hand, showed 12 pairs. LONGLEY (1927) and RANDOLPH (1928) in recent investigations have extended considerably our knowledge of the behavior during meiosis and the distribution through gametes of such "extra" chromosomes.

It is possibly unjust to restrict the term triploid to those 3n forms which show trivalents during meiosis. However, in the 3n Zea plants showing 10 trivalents, behavior during meiosis can be compared directly with other 3n forms which show trivalents. The literature on such types is growing rapidly. Under the heading of triploids showing trivalents would come some individuals of Morus (OSAWA 1920), Canna (BELLING 1921, 1925), Datura (BELLING et al.), Hemerocallis (BELLING 1925), Hyacinthus (BELLING 1925), Campanula (GAIRDNER 1926), Lycopersicum (MANN-LESLEY 1926) Zeax Euchlaena F_1 (LONGLEY 1924; Randolph unpublished), Drosophila (METZ 1925; MORGAN 1925; BRIDGES 1921, 1922, 1925) and Zea.

The genetics of triploids has been followed in the case of Datura, Drosophila and Lycopersicum. For the literature on trisomic inheritance, see discussion under Genetic Investigation.

MATERIAL AND METHODS

The behavior of chromosomes during meiosis, exclusive of the early prophase stages, has been studied in many diploids, two triploids and 50 F_1 individuals resulting from direct and reciprocal crosses made between diploid individuals and the triploid plant (see table 1). Studies were made only on microsporcytes.

For the study of chromosome number and behavior during meiosis the iron-aceto-carmine method was found to be very serviceable. In some cases the smears were dried slowly and then mounted in balsam. Photomicrographs 14 and 15, in plate 6 and text figure 7 were made from preparations seven months old which had been so treated. In some cases where the stain was too heavy it was extracted in a weak solution of acetic acid, the whole slide being warmed to hasten the extraction.

Other sporocyte material and root tips were fixed in a Bouin solution modified according to Allen, mounted in paraffin and sectioned 10-15 microns thick. Heidenhains iron-alum-haematoxylin and Flemming's triple stain were used.

The photomicrographs were taken with the aid of a Leitz "Makam" camera, an 8× periplanatic ocular, and a 1.8mm, N.A.1.25 Spencer achromatic objective. A Wratten green filter, number 56, was used to increase definition.

A BRIEF RÉSUMÉ OF MEIOTIC BEHAVIOR IN DIPLOID INDIVIDUALS

At diakinesis the diploid individuals show characteristically 10 pairs of chromosomes. At the time the nuclear membrane disappears the bivalent nature of the chromosomes becomes relatively indistinct because the chromosomes are so contracted and closely appressed. However, after the bivalent chromosomes line up at the equator and show evidence of commencing anaphasic disjunction, characteristic forms are assumed (see bivalent chromosomes in metaphase figures in plates 2-5).

The double (dyad) nature of each chromosome of the pair is clearly indicated at early anaphase, frequently before the chromosomes have completely disjoined. It is evident from a study of metaphase *I*[1], early anaphase *I*, and metaphase *II* that the spindle fiber attachment is either sub-terminal, sub-median or median.

In consequence of partial anaphasic separation of members of the dyads the chromosomes appear as double Vs (median or sub-median attachments, or as almost single Vs (sub-terminal attachments). This picture varies considerably in different anaphases due to the unusual compactness of the dyads in some figures and to their loose, long-armed form in other figures (figure 34). In the latter case one or more arms of a dyad may become much pulled out, remaining near the equator during the continued poleward advance of the spindle attachment part of the chromosome. The telophase passes over into a more or less typical interphase wherein the individual chromosomes lose their observable identity. A cell plate, apparently initiated by the phragmoplast, is evident at this time.

From the very earliest prophase of *II* in which the chromosomes can be recognized as distinct bodies, their form is very characteristically that of an H or an X. Contraction of the chromatic material continues until the chromosomes become relatively small Hs and Xs (figure 31). The nuclear membrane shrinks about the chromosomes with the formation of a multipolar spindle. This, in turn, passes into a bipolar spindle with the disappearance of the nuclear membrane. The shape of the metaphase *II* chromosomes depends greatly upon the amount of contraction of the chromosomes at this time. In some cases the dyads appear as relatively long, double threads. On the other hand, if contraction of the chromosomes

[1] The first meiotic mitosis will be designated as *I*, the second meiotic mitosis as *II*.

continues, many of these dyads appear as double 'dumb-bells", the dumb-bell shape probably being due to an accentuated constriction (see SAKAMURA 1920; GOTOH 1924). Apparently the anaphase *II* chromosomes derive their varied shapes, such as bent rods in some sporocytes, or dumb-bell-like bodies in others, from the state of contraction of the chromosomes at metaphase *II*. As a result of division *II* a quartet of spores is formed. Cell plate formation apparently initiates wall formation as has been shown by REEVES (1928).

In most cases meiosis is thus characteristically regular, but several types of behavior having much theoretical interest have been observed. Occasionally and sometimes rather frequently in certain diploid cultures a pair of chromosomes showed non-conjugation as described by BELLING (1925) and others. Many of the diakinesis figures within an anther contained distinctly 10 bivalent chromosomes, while other sporocytes within the same anther showed $9^{II}+2^{I}$.[2] Whether there was never any meiotic association of the 2 homologous chromosomes or whether the disassociation of a synapsed pair to form 2 univalents took place in late prophase *I* is unknown. Neither is it known whether the same homologous pair was concerned each time a figure with $9^{II}+2^{I}$ was observed. The cause of this appearance is certainly not a lack of homology of the chromosomes in all cases (RANDOLPH 1928).

Other irregularities such as non-disjunction of a pair of chromosomes, lagging and splitting of univalents at *I*, two-nucleated sporocytes, metaphase *I* figures with more or less than the expected chromosome number, and metaphases and anaphases with multiples of the diploid chromosome number have been observed in diploid individuals.

MEIOSIS IN THE TRIPLOID INDIVIDUAL

A brief description of meiosis in a triploid plant has been given (RANDOLPH and MCCLINTOCK 1926); therefore certain features stressed in that paper will not be redescribed here.

Diakinesis stages in aceto-carmine in the triploid gave far better results than similar stages in fixed and sectioned material. In the former method the synaptic condition, that is, whether trivalent, bivalent, or univalent, of all the chromosomes in a single nucleus usually could be determined (figure 1),[3] whereas, in the section only a few of the chromosomes within

[2] Bivalents will be designated by the exponent Roman numeral II; univalents, exponent I; trivalents, exponent III, etc.

[3] Figures in plates 1–5 are numbered from 1–47. The photo-micrographs in plate 6 are numbered 1–15. To avoid confusion, "plate 6" will always be added to the figure number when reference is being made to the photo-micrographs.

Explanation of Plates

Figures in plates 1–5 were made with the aid of an Abbe camera lucida. Approximate magnifications:

Figures 1–24, 26–29, 32–46: 1200x.

Figures 25, 30, 31: 500x.

Figure 47: 700x.

Figures in plates 1 and 6 are from aceto-carmine smears and sectioned material. Figures in plates 2–5 from aceto-carmine smears only. The small numbers at left of figures indicate the culture and plant number.

Plate 1.

Figure 1.—Diakinesis in microsporocyte of triploid (56_2), showing 10 trivalents. Aceto-carmine.

Figure 2.—Diakinesis in triploid, showing 9 trivalents, 1 bivalent and 1 univalent. Sectioned material.

Figure 3.—Metaphase *I* in triploid, showing 10 trivalents. Aceto-carmine.

Figure 4.—Metaphase *I* in triploid. 10 trivalents. Sectioned material.

Figure 5.—Metaphase *I* in triploid. 9 trivalents, 1 bivalent and 1 univalent; univalent preparing to split and separate while trivalents and bivalents disjoin. Sectioned material.

Figure 6.—Metaphase *I* in triploid. 7 trivalents, 3 bivalents and 3 univalents. 2 univalents in the spindle region nearer the lower pole, 1 in the cytoplasm outside the spindle. Sectioned material.

Figure 7.—Metaphase *I* in triploid. 8 trivalents, 1 bivalent and 1 univalent in main spindle; 1 trivalent in separate spindle running perpendicular to plane of section. See photograph of same: plate 6, figure 5.

Figure 8.—Anaphase *I* in triploid. 15 chromosomes going to each pole. Aceto-carmine.

Figure 9.—Anaphase *I* in triploid. 14 dyads at each pole; 2 lagging and separating in center of figure. Sectioned material.

Figure 10.—Anaphase *I* in triploid. 12 dyads in upper pole, 11 in lower pole and 7 separating dyads in center of spindle. See photograph of same plate 6, figure 6. Sectioned material.

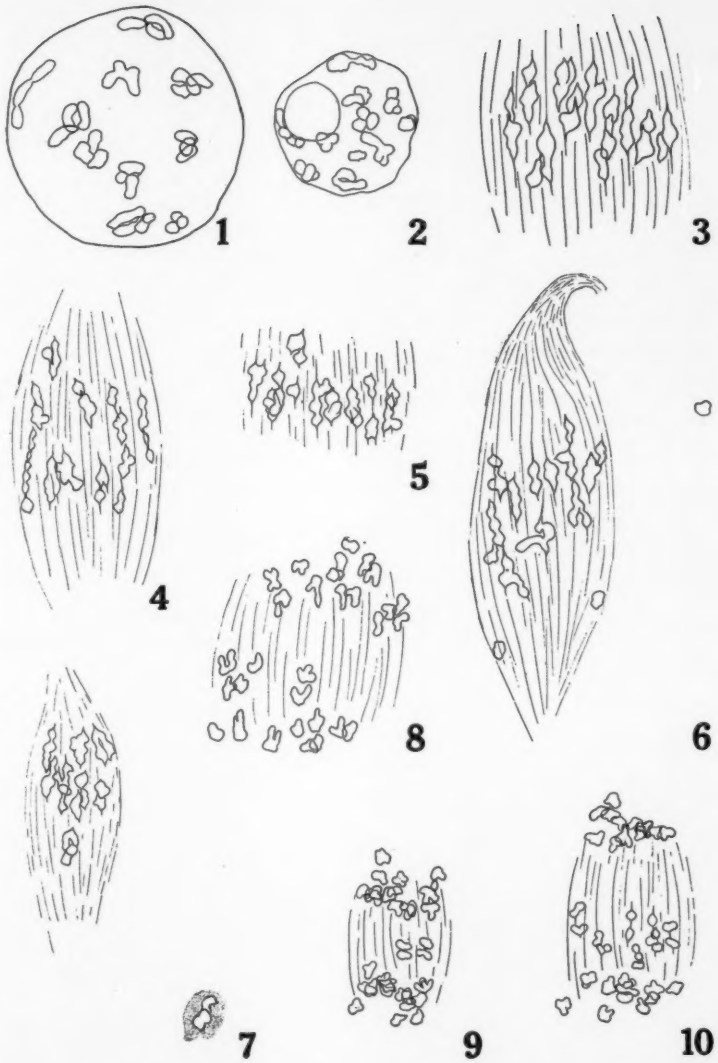
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PLATE 2

Figures from 2n+1 F_1 plants.

FIGURE 11.—Metaphase *I* showing $9^{II}+1^{III}$.

FIGURE 12.—Metaphase *I* from same plant showing $10^{II}+1^{I}$. The univalent is lying in the equatorial plate.

FIGURE 13.—Same as figure 12, only the univalent is lying toward the upper pole.

FIGURE 14, 15, and 16.—Metaphase *I* trivalents taken from different $9^{II}+1^{III}$ figures arranged to show the various appearances of the same trivalent in different cells and the trivalents from different 2n+1 plants.

FIGURE 17.—Metaphase *I* in a 2n+1 plant, showing the double number of chromosomes ($20^{II}+2^{I}$) in one figure.

FIGURE 18.—Metaphase *I* spindle with four times the normal number of chromosomes: $40^{II}+4^{I}$.

FIGURE 19.—Three connecting metasphase *I* spindles in a plasmodial mass.

FIGURE 20.—Anaphase *I* figure in a 2n+1 plant, showing fragmentation of chromosomes in progress.

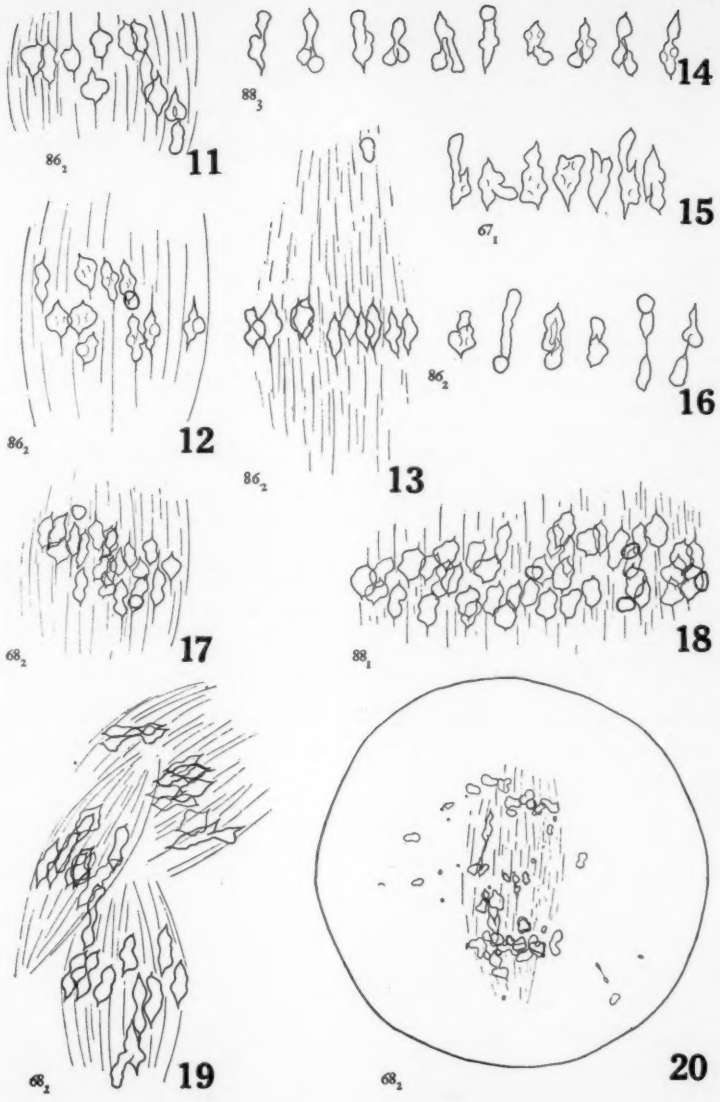
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PLATE 3

Figures from 2n+2 F_1 plants.

FIGURE 21.—Diakinesis: $9^{II}+1^{III}+1^{I}$.

FIGURE 22.—Late metaphase *I*: $8^{II}+2^{III}$. The members of the trivalent to the right have nearly disjoined.

FIGURE 23.—Metaphase *I*: $10^{II}+2^{I}$.

FIGURE 24.—Anaphase *I*: 10 dyads in upper pole, 11 toward lower pole, 1 dyad with separating halves at center of spindle.

FIGURE 25.—Microspore quartet with unequal spores.

FIGURE 26.—Metaphase *I*: $7^{II}+2^{III}$. This figure contains 1 bivalent less than most of the sporocytes in this plant.

FIGURE 27.—Metaphase *I*: $4^{II}+6^{IV}+2^{VI}$. 2 large sexivalents above and below plate region; counting from the right in region of plate, the second, third, fourth, sixth, seventh and eighth. groups are quadrivalents, the remaining 4 chromosomes are bivalents.

FIGURE 28.—Anaphase *I* in same plant with the double number of chromosomes.

FIGURE 29.—Anaphase *II*: 12 monads at each pole.

FIGURE 30.—Microspore quartet resulting from *I* and *II*. Note chromatic bodies in cell plate region.

FIGURE 31.—Prophase *II*: 10 dyads in cell to left, 12 dyads in cell to right.

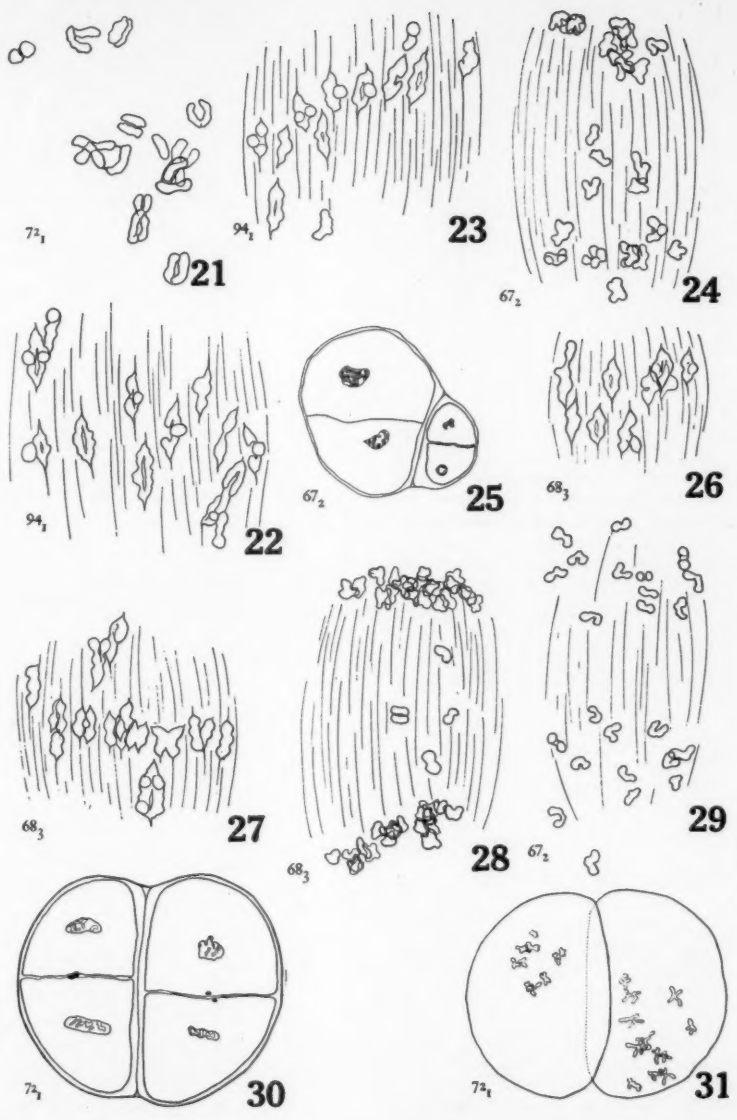
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PLATE 4

Figures from 2n+3 to 3n F_1 individuals.

FIGURE 32.—Metaphase *I*: $7^{II}+3^{III}$.

FIGURES 33.—Metaphase *I*: $8^{II}+2^{III}+1^{I}$.

FIGURE 34.—Anaphase *I* in 2n+3 individual: 10 dyads toward upper pole, 11 towards lower pole, 2 splitting at center of spindle.

FIGURE 35.—Metaphase *I*: $6^{II}+4^{III}$.

FIGURE 36.—Metaphase *I*: $8^{II}+1^{III}+4^{I}$.

FIGURE 37.—Metaphase *I*: $6^{II}+4^{III}$. See photograph of same, figure 14, plate 6.

FIGURE 38.—Metaphase *I*: $3^{II}+7^{III}$. Members about to disjoin in 2 trivalents at left of figure.

FIGURE 39.—Metaphase *I*: $6^{II}+4^{III}$.

FIGURE 40.—Metaphase *I*: 10^{III}.

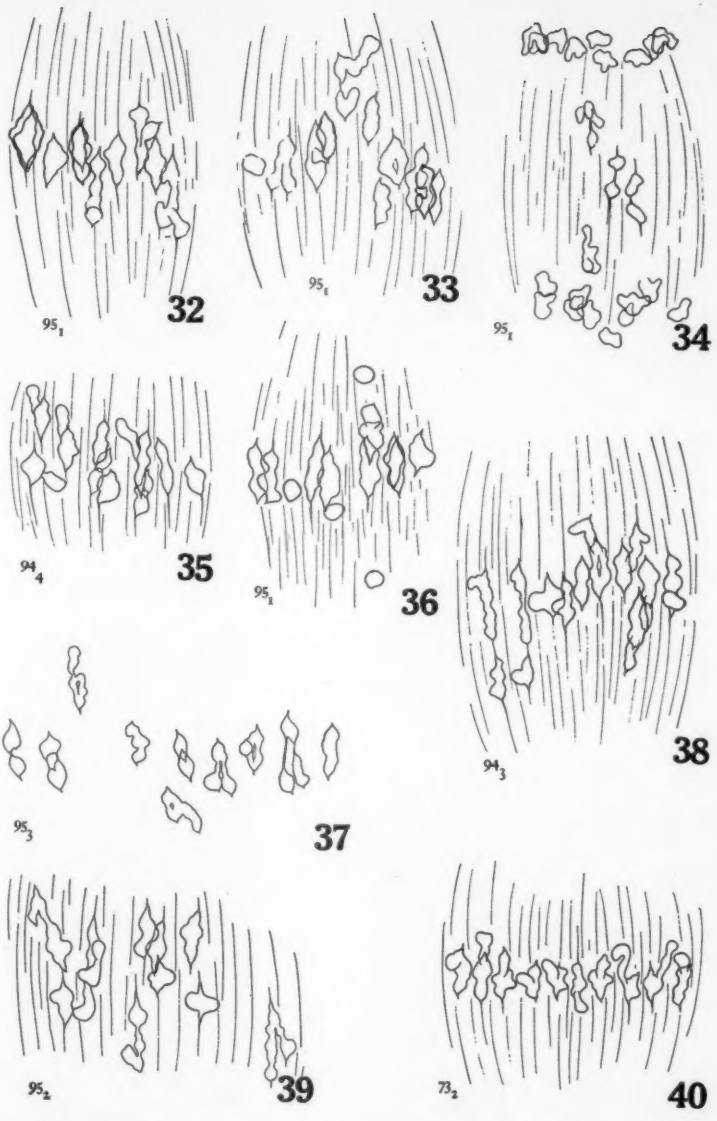
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PLATE 5

All figures are from plant 94_3, 2n+5.

FIGURE 41.—Metaphase *I*: $5^{II}+5^{III}$.

FIGURE 42.—Metaphase *I*: $9^{II}+7^{I}$.

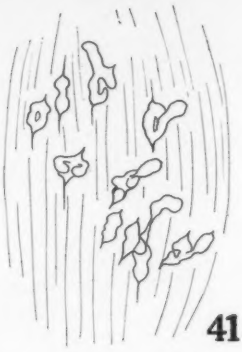
FIGURE 43.—Diakinesis: $4^{II}+3^{III}+8^{I}$.

FIGURE 44.—Division *I*: 25^{I}.

FIGURE 45.—Anaphase *I*: 10 univalents at upper pole; 11 at lower pole; $1^{II}+2^{I}$ at center of spindle.

FIGURE 46.—Early anaphase *I*: $1^{II}+1^{III}+20^{I}$.

FIGURE 47.—Showing production of 6 unequal spores from a single microsporocyte.



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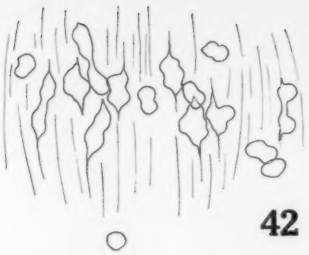

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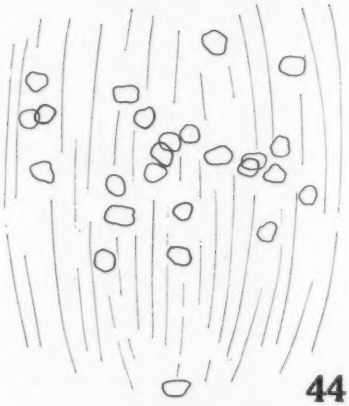

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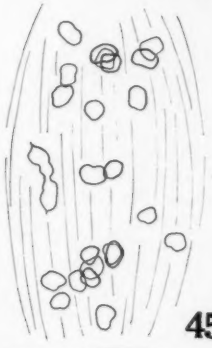

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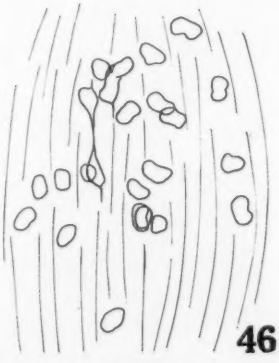

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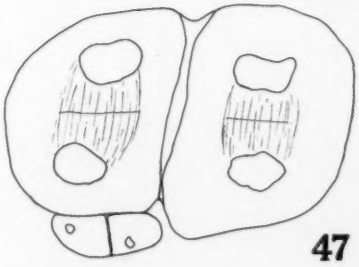

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PLATE 6

Figures 1–13 are of sectioned and haematoxylin stained material. Figures 14 and 15 are of ceto-carmine smears. Magnification, approximately 680x.

FIGURE 1.—Diakinesis in triploid (56_2). One trivalent visible.

FIGURE 2.—Late metaphase *I* in triploid showing disjunction of 3 members of a trivalent.

FIGURE 3.—Same sporocyte as in figure 2, showing another trivalent.

FIGURE 4 —Metaphase *I* in sporocyte of triploid, showing 3 trivalents in same focus.

FIGURE 5.—Metaphase *I* with 2 spindles. Small spindle to right of cell runs perpendicular to the plane of the section and contains one trivalent. See drawing of same in plate 1, figure 7.

FIGURE 6.—Anaphase *I* showing lagging and separation of halves of split univalents. Same as figure 10, plate 1.

FIGURE 7.—Prophase *II* in triploid. Spindles are forming about both dwarf and major nuclei.

FIGURE 8.—Telophase of *I*; two large cells and one small cell. The nuclei of the two large cells are not in focus.

FIGURE 9.—Telophase of *II* resulting from a tripolar spindle.

FIGURE 10.—Formation of "dwarf" nuclei from prophase *I* nucleus. Arrow 1 points to 3 small nuclei in row. Arrow 2 points to a larger nucleus beside major nucleus.

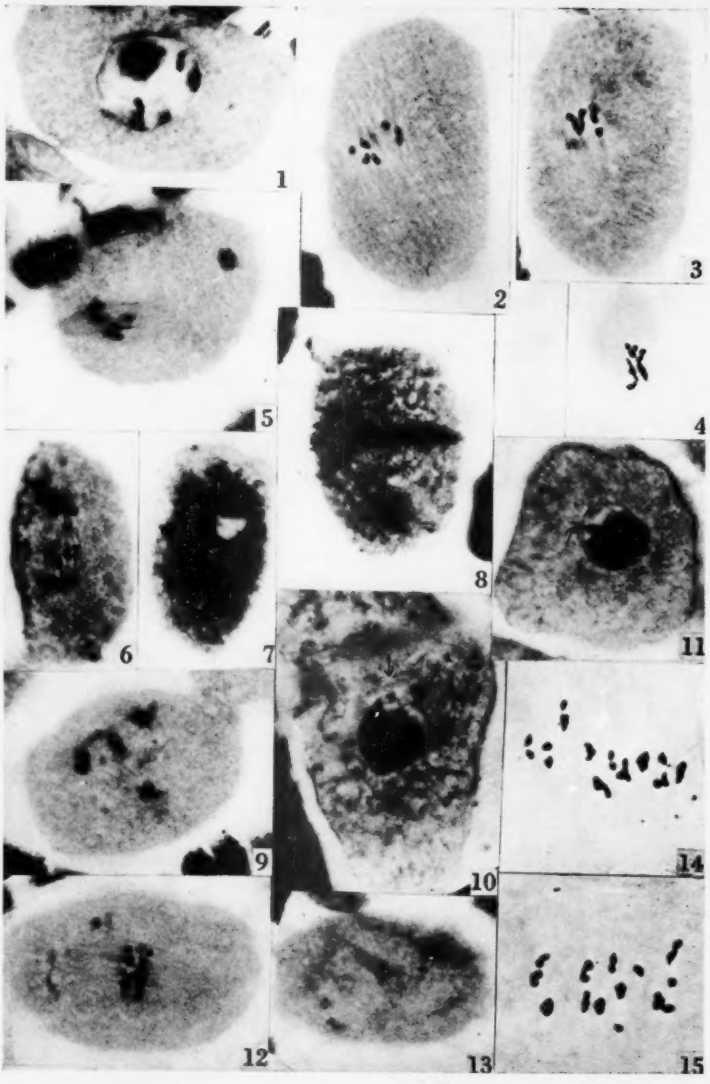
FIGURE 11.—Prophase *I*. A chromatic thread apparently projecting through the nuclear membrane into the cytoplasm.

FIGURE 12.—Early anaphase *II* in triploid. Sporocyte possessing but one spindle containing the double number of chromosomes.

FIGURE 13.—Metaphase *II* in triploid showing several spindles, variously oriented, with few chromosomes in each.

FIGURE 14.—Metaphase *I* in 2n+4 individual (95_3) showing $6^{II}+4^{III}$. Same as figure 37, plate 4.

FIGURE 15.—Metaphase *I* in adjacent sporocyte to above showing $10^{II}+4^{I}$.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a nucleus could be easily interpreted as a general rule. Occasionally all the chromosomes in a single nucleus could be well interpreted (figure 2, plate 1; figure 1, plate 6).

In many cases the chromosomes at diakinesis are arranged in ten groups of three each (figure 1). A definite and typical association of any 3 homologues, giving a constant, morphologically recognizable trivalent, apparently does not exist. This is more clearly evident in F_1 individuals in which the single extra chromosome forms one trivalent, the different figures of which are directly comparable (see plate 2, figures 14, 15 and 16). We see here that the same trivalent both in diakinesis and metaphase *I* assumes various forms which are undoubtedly due to the relative closeness and type of approximation of the three members.

Figure 2 illustrates an apparent tendency on the part of one of the homologous chromosomes to disassociate from the other two. In diakinesis all stages of approximation of the third chromosome are seen: a group of three equally spaced chromosomes; a group which would definitely be recognized as a bivalent with an attached univalent; a bivalent with the univalent rather distantly attached by a fine thread; and finally, an apparently complete disassociation of the univalent from the bivalent. It is obvious, therefore, that although distinct univalents are evident at diakinesis, this by no means indicates that synapsis among the three homologues had not taken place at an earlier prophase stage.

FIGURE 1.—Polar view of somatic metaphase from root tip of triploid plant. $3n=30$.

As has been stated, the proportion of trivalents, bivalents and univalents varies in adjacent sporocytes. The presence of 9 trivalents, 1 bivalent and 1 univalent was most usual, those with 2, 3 and 4 univalents being less frequent. We observed in no case more than 4 univalents, that is, $6^{III}+4^{II}+4^{I}$. It is not at all inconceivable, from what is known of the F_1 individuals, that greater disassociation took place but was unobserved in the material examined.

Again, at metaphase *I* we find, very frequently, 10^{III} (figures 3 and 4). However, as in diakinesis, there are commonly present fewer trivalents with a consequent increase in bivalents and univalents (figures 5 and 6).

Sometimes no equatorial plate was formed, the metaphase *I* chromosomes being distributed along the length of the spindle (figures 4 and 6).

In position and behavior the univalents vary considerably. Sometimes they appear in or near the plate. Here they are easily distinguishable because of their smooth contour and their round or oblong shape. However, very occasionally, a univalent lying in the plate exhibits distinct spindle fiber attachments (figure 5). Here the univalent is probably preparing to divide at the same time that the trivalents and bivalents are about to disjoin. This is in contrast to the usual appearance of the separation of the members of a dyad in *I* which takes place in lagging chromosomes after disjunction of the members of the trivalents and bivalents. Sometimes the univalents lie within the spindle area some distance from the plate on either side.

It is not uncommon to find one or more univalents lying in the cytoplasm apart from the spindle (figure 6). Occasionally a sporocyte was observed with one trivalent lying in a separate spindle distinctly apart from the main metaphase *I* spindle (figure 7, and photograph of same, plate 6, figure 5). In other sporocytes the minor spindle was connected with the major spindle.

The distribution of the chromosomes in anaphase *I* appears to be a random one. In many anaphase *I* figures the chromosomes passed to the poles without irregularities (figure 8). However, in many other figures marked irregularities were obvious, such as lagging of dyads, lagging and splitting of dyads (figures 9, 10; figure 6, plate 6) and splitting and partial separation of a dyad which had commenced to pass to one pole. Other irregularities, such as the fragmentation of part of a dyad as it passed to the pole, were numerous in some sporocytes of certain anthers. The extent of fragmentation within a sporocyte varied from a part of a single dyad to practically the whole complement.

In consequence of the many irregularities observed in diakinesis, metaphase *I* and anaphase *I*, the appearance of the sporocytes in telophase and interkinesis is diverse. In some we find two nuclei of approximately equal size between which a cell plate has formed. In figure 8, plate 6 is shown the formation of three distinct nucleated protoplasmic masses separated by cell plates formed in division *I* (nuclei of two larger protoplasmic masses not in focus). In other cases one or more small nuclei are not set off by cell plates from the major nuclei. It is conceivable that the condition depicted in figure 8, plate 6 resulted from a two-spindle sporocyte as in figure 5, plate 6, in which a minor spindle connects at right angles with the major spindle. In the other cases, however, the small nuclei may have

originated from univalents which lagged during anaphase *I*. Some sporocytes showed a great many nuclei grading in size from large to very minute. The very small nuclei probably represent reorganized chromatin fragments, since breaking up of the chromosomes during anaphase *I* is so obvious in some cells.

During interkinesis the dyad and monad chromosomes become invisible as such and the nucleus takes on a metabolic appearance. As the prophase *II* progresses the characteristic X-, H- or rod (monad)-shaped chromosomes become evident. Frequently, in those cells which possess small nuclei besides a large one, a similar prophase *II* transformation appears to be going on within these small nuclei. Such is the case shown in figure 7, plate 6. The small nucleus, about which a spindle is forming, contains a dyad chromosome. Consequently more than two metaphase *II* spindles may occur within a single sporocyte (figure 13, plate 6). In both acetocarmine and sectioned material sporocytes were seen with all the chromosomes in a single metaphase *II* spindle (figure 12, plate 6).

In general, the second meiotic mitosis appears much freer from irregularities than the first. There is very little fragmentation and comparatively little lagging of chromosomes. In many cases the wall laid down during *I* disappears soon after its formation. In consequence, the spindles of *II* lie in a common mass of cytoplasm. Rather infrequently tripolar spindles were observed which had been formed from a partial fusion of the two metaphase *II* spindles (figure 9, plate 6).

In consequence of the irregularities attending division *I* and *II* a single sporocyte can produce from two to many spores, the nuclei of which contain various amounts of chromatin. These spores, in turn, may be uninucleate or, as is frequently the case, multinucleate, the nuclei being variable in size and probably in chromosome content. The pollen grains exhibit such size relationships as would be expected from the above description.

PLASMODIAL SPOROGENOUS TISSUE

In the triploid plant and its F_1 descendants the sporocytes within some anther loculi, instead of existing as well defined individual cells, showed various degrees of cytoplasmic fusion. All conditions from a partial union of a few adjacent cells to a complete loss of identity of the sporocytes were observed, the result in the latter case being a plasmodium which filled the entire anther cavity. In some plasmodia two or more nuclei were so close together that only a very thin layer of cytoplasm appeared to exist between them. In several instances two adjacent nuclei appeared to be fusing.

The nuclei in the plasmodium continue their prophase meiotic development, forming well defined diakinesis and metaphase *I* figures (text figure 2). Probably as the result of the closeness of nuclei in these plasmodial masses, metaphase *I* spindles connect in various ways. It is conceivable that some of the metaphase *I* spindles exhibiting a double number of chromosomes have arisen in this manner. It is also conceivable that other metaphase *I* spindles possessing the double number of chromosomes, the homologous members of which, however, show a close synaptic attraction, have arisen by an earlier prophase fusion of nuclei, or possibly even by a pre-meiotic fusion.

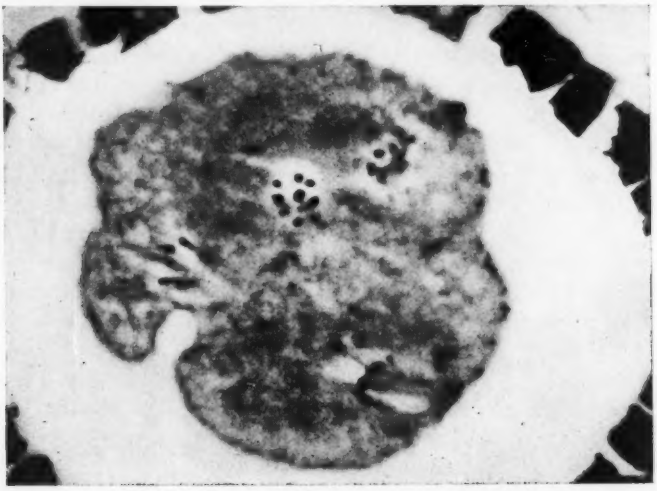

FIGURE 2.—Photomicrograph of plasmodium in anther showing 3 metaphase *I* spindles and one nucleus in late diakinesis.

As a result of the first meiotic division the plasmodium becomes divided into smaller masses separated by well defined cell plates.

CHROMATIN EXTRUSION IN SPOROGENOUS TISSUE

The breaking up of chromatin to form many small nuclei which become distributed in the cytoplasm was seen to occur in several stages of meiosis.

Sporocytes in prophase *I* exhibited numerous figures of "cytomixis" as described by many authors. Another striking prophase *I* phenomenon was the presence of small supernumerary nuclei distributed in the cytoplasm or closely associated with the main nucleus (figure 10, plate 6). It is pos-

sible, in some cases, that the small nuclei were formed from the main nucleus by budding (see SAKAMURA 1920). In other cases chromatin threads which have been seen extending through the nuclear membrane into the cytoplasm (figure 11, plate 6) may have given rise to some of these diminutive nuclei.

Extensive chromosome fragmentation occurring during anaphase *I* was observed in a number of sporocytes. Many small nuclei were observed in some sporocytes which obviously had been formed from reorganized chromosome fragments.

THE F_1 GENERATION

Because of the observed cytological behavior in the triploid individual, it was of great interest to follow the distribution of the chromosomes through future generations. Also, as little was known concerning the genetic constitution of the three chromosomes of an homologous group, crosses were made to determine this to some extent. In almost every case where crosses were made the chromosome number and meiotic behavior of the plants used had been determined (see table 1). This was obviously necessary, as LONGLEY (1927) and RANDOLPH (1928) have shown that in many genetic cultures one or more extra chromosomes are present which are carried on to future generations.

Further indication that the extra chromosome in the F_1 individuals originated from the gametes in the triploid was seen in the contrast between the appearance and behavior of these F_1 individuals with extra chromosomes and in those extra chromosome individuals within genetic strains of maize. The extra chromosomes (when more than one extra chromosome is present it seems to be due to a reduplication of the same chromosome) running through some genetic strains of maize and found principally in Black Mexican sweet corn do not affect size, vigor or fertility to any noticeable degree. They are carried through in the eggs or the pollen. The extra chromosomes in the F_1 plants of the cross triploid $\times$ diploid, on the other hand, affect size, vigor and fertility and are carried only to a small extent through the pollen. In a $2n+1$ plant of Black Mexican the chromosomes do not, so far as I am aware, form $9^{II}+1^{III}$, but always $10^{II}+1^{I}$. This is quite in contrast with the $2n+1$ F_1 plants from the cross diploid $\times$ triploid, which form most frequently $9^{II}+1^{III}$. On the basis of studies of chromosome morphology made by Doctor RANDOLPH in extra chromosome plants of Black Mexican sweet corn and in some genetic strains of maize, it is believed that this extra chromosome is not a whole duplicate of any one member of the haploid set of ten chromosomes. On the other hand, the

TABLE 1

CROSS, 1925	CHROMOSOME NUMBER OF ♀ PARENT	CHROMOSOME NUMBER OF ♂ PARENT	NUMBER OF KERNELS ON EAR	CHARACTER OF F_1 KERNELS	F_1 CULTURE NUMBER		NUMBER OF KERNELS PLANTED	NUMBER OF PLANTS DEVELOPED	CHROMOSOME NUMBER OF F_1 PLANTS											
					GREENHOUSE	FIELD			2n	2n+1	2n+2	2n+3	2n+4	2n+5	2n+6	2n+7	2n+8	2n+9	3n	$11^{II}+2n+2$
56_2+	10^{III}	10^{III}	6	1 purple sugary 3 colorless sugary 2 underdeveloped		96 97	6	0	..	..	..	..	..	..	..	..	..	..	..	..
$56_2 \times 33_6$ *AB p_1 l_a jV*	10^{III}	10^{II}	34	8 purple starchy 26 colorless starchy	68 69	94 95	31	11	..	1	3	1	3	1	..	1	..	..	..	..
$56_2 \times$E14–871_6	10^{III}		10	4 colorless starchy 6 poorly developed	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
56_2 kernels formed in main and tiller tassels.	10^{III}	?	22	9 purple starchy 4 colorless starchy 2 colorless sugary 7 underdeveloped	64 65 66 67	..	7	3	..	1	1	1	..	..	..	..	..	..	..	..
$2_7 \times 56_2$ *A b p_1*	?	10^{III}	0		..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
$16_3 \times 56_2$ *a C R $S_u W_x$* $l_a y$ b p_1	12^{II}	10^{III}	0		..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
$17_2 \times 56_2$ *AC r S_u b p_1*	10^{II}	10^{III}	17	9 purple starchy Y 8 colorless starchy Y	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
$20_{12} \times 56_2$ *A P l_a*	?	10^{III}	0		..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
$21_{14} \times 56_2$ *ACR p_r b p_1*	10^{II}	10^{III}	1	1 purple starchy	..	81	1	1	1	..	..	..	..	..	..	..	..	..	..	..
$23_9 \times 56_2$ *a b p_1 C R*	10^{II}	10^{III}	9	4 purple starchy Y 5 colorless starchy Y	77	82 83	8	6	6	..	..	..	..	..	..	..	..	..	..	..
$24_{13} \times 56_2$ *Ac R b p_1*	10^{II}	10^{III}	6	4 purple starchy 1 colorless starchy 1 outside pollination	78	84	4	4	3	..	..	..	..	..	..	..	..	..	..	1
$25_1 \times 56_2$ *ACr s_a*	13^{II}	10^{III}	2	2 colorless, underdeveloped	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
$26_1 \times 56_2$	10^{II}	10^{III}	22	9 purple starchy 13 colorless starchy	73 74	85 86	13	8	6	1									1	..
Abp l_a y	10^{II}	10^{III}	0		..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..

$25_1 \times 56_2$ *ACr* s_u	13^{III}	10^{III}	2	2 colorless, underdeveloped	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
$26_1 \times 56_2$	10^{II}	10^{III}	23	9 purple starchy 13 colorless starchy	73 74	85 86	13	8	6	1									1	..
$27_1 \times 56_2$ *Abp*$_1$ $l_g y$	10^{II}	10^{III}	0		..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
$28_4 \times 56_2$ *Abp*$_1$ l_g	10^{II}	10^{III}	0		..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
$30_{14} \times 56_2$ *Abp*$_1$ z_u *j*	?	10^{III}	1	1 purple underdeveloped	..	87	1	0	..	..	..	..	..	..	..	..	..	..	..	..
$31_1 \times 56_2$ *j* l_g	?	10^{III}	0		..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
$33_5 \times 56_2$ *AB*p_1 $l_g j$ S_u *Y*	10^{II}	10^{III}	7	3 yellow starchy 4 badly infected, broken kernels	70	93	3	2	2	..	..	..	..	..	..	..	..	..	..	..
$34_{22} \times 56_2$ Anther ear	?	10^{III}	3	3 yellow starchy	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
$36_2 \times 56_2$ *AcR* s_u *b* p_1 *y*	10^{II}	10^{III}	49	19 purple sugary 30 colorless sugary	71 72	88 89	20	10	3	5	2	..	..	..	..	..	..	..	..	..
$38_3 \times 56_2$ *ACr g b* p_1	10^{II}	10^{III}	18	4 purple starchy 14 colorless starchy	75 76	90 91	8	6	6	..	..	..	..	..	..	..	..	..	..	..
$44_{12} \times 56_2$ gl_1	?	10^{III}	0		..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
$45_3 \times 56_2$ gl_3	10^{II}	10^{III}	10	4 purple starchy Y 6 colorless starchy Y	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
$51_2 \times 56_2$ *b, P*^{rr}	?	10^{III}	15	Red pericarp, starchy 5 purple, 10 colorless	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
E13–533×56_2		10^{III}	6	6 teosinte-like	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
E14–$871_2 \times 56_2$		10^{III}	1	1 teosinte-like	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
$R191_1 \times 56_2$		10^{III}	13	13 purple starchy Y	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
$R251_2 \times 56_2$		10^{III}	3	3 yellow sugary	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
$R250_2 \times 56_2$		10^{III}	2	2 purple sugary	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
$R251_2 \times 56_2$ tiller		10^{III}	1	1 colorless sugary Y	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..

* See note in table 2

extra chromosome in the 2n+1 F_1 individuals of the cross diploid × triploid is a true duplicate of one member of the haploid set. The same chromosome is very likely involved in all the usual 2n+1 plants of Black Mexican sweet corn and genetic strains of maize. The extra chromosome in the several 2n+1 F_1 individuals of the cross diploid × triploid is not always the same, but represents different members of the haploid set.

Table 1 was constructed to show what crosses were made and the general results obtained. All the available material resulting from these crosses has not yet been investigated, but a sufficient amount has been examined to give an indication of what, in general, has taken place.

As it was desired to obtain the best results with the least amount of pollen, care was taken to collect the pollen soon after shedding. In every cross sufficient pollen was used to pollinate a whole ear in an ordinary maize plant. As the triploid plant had several good tillers, much pollen was available even though many anthers never opened. The fact that only a few kernels developed on an ear as a result of these crosses could not, then, be attributed to a lack of sufficient pollen.

The occurrence of a selection of gametes containing certain chromosome complements (in the cases where triploid pollen was used in crossing on to diploid plants) was markedly indicated by the few kernels on an ear and the chromosome number shown by the individuals coming from these kernels (table 5). Where there are few kernels on an ear the 10-chromosome carrying gametes apparently have been successful, whereas the extra-chromosome carrying gametes probably have not functioned in fertilization. In other crosses where many kernels were found on an ear ($36_2 \times 56_2$, table 5) not only the 10-chromosome carrying gametes functioned but also many of the 10+1 and 10+2-chromosome gametes. Why it is that in all cases where a sufficient amount of pollen was used, there appeared such a dissimilar selectivity toward certain chromosome-carrying gametes among the different plants, is not known.

When the cross triploid ♀ × diploid ♂ is made, the results are strikingly different (table 5). Here, apparently, the eggs containing various chromosome numbers function. However, they do not function perfectly, as relatively few kernels developed on an ear. Since microsporogenesis is known to produce such varied types of cells with regard to chromosome complement, it is conceivable that megaspores in many ovules are incapable of developing a functioning gametophyte. The plants which were least vigorous contained the highest number of extra chromosomes. These grew from small underdeveloped kernels which had been carefully treated to induce germination. As there were many of them on the ear, it is probable

TABLE 2

Culture numbers of F_1 individuals with their respective chromosome number.

10^{II}	$9^{II}+1^{II}$	$8^{II}+2^{III}$	$7^{II}+3^{III}$	$6^{II}+4^{III}$	$5^{II}+5^{III}$	$4^{II}+6^{III}$	$3^{II}+7^{III}$	$2^{II}+8^{III}$	$1^{II}+9^{III}$	10^{III}	11^{II*}
73_1	67_1	67_2	65_1	94_4	94_2		94_3			73_2	84_2
74_1	68_2	$68_{3,4}$	95_1	$95_{2,3}$							
75_1	$72_{2,3}$	72_1									
$76_{1,2}$	86_2	88_4									
$77_{1,2}$	$88_{1,3}$	94_1									
$78_{1,2}$	89_1										
81_1											
$82_{1,2,3}$											
83_1											
84_1											
$85_{1,2,3}$											
86_1											
$88_{2,3}$											
89_2											
90_1											
$91_{1,2}$											
$93_{1,2}$											

* This individual probably came from an outside pollination. The two extra chromosomes are similar to those found in extra chromosome plants of Black Mexican sweet corn.

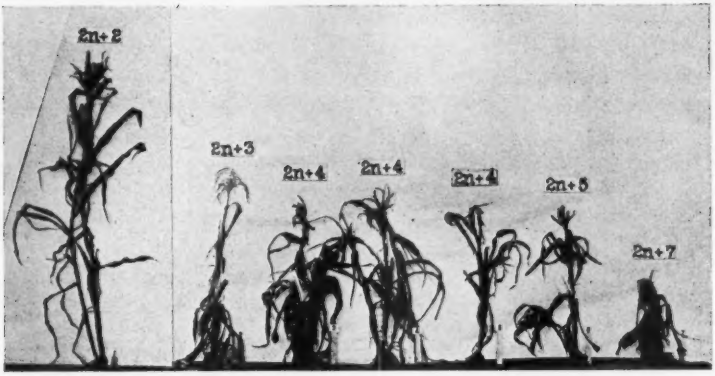

FIGURE 3.—Photograph of F_1 plants grown in the field resulting from the cross triploid ♀ × diploid ♂ arranged according to chromosome complement. Plant no. 1, 94_1; no. 2, 95_1; no. 3 95_2; no. 4, 95_3; no. 5, 94_4; no. 6, 94_2; no. 7, 94_3.

that if all could have been induced to germinate a wider range of chromosome number in the F_1 would have been found. Thus the limiting factor in

FIGURE 4.—Photograph of F_1 plants from reciprocal cross (diploid ♀ × triploid ♂) to that shown in text figure 3. Both individuals were diploid. Plant no. 8, 93_2; no. 9, 93_1.

some cases is probably not the inability of the female gametophyte to function, but rather the capacity of the chromosome complement within the embryo to participate in growth and development.

The marked effect of the presence of extra chromosome on the appearance of the plant is graphically represented by text figures 3, 4, 5 and 6. These photographs were made after the plants had matured ears. In consequence the leaves are dried and curled. However, the relative sizes of the plants containing varying chromosome numbers are preserved.

Two plantings of F_1 kernels were made, one in the spring of 1926 in the greenhouse and one in the summer of 1926 in the field. For a summary of germination results see table 3.

TABLE 3

Results of germination.

Origin of F_1 kernels	GREENHOUSE		FIELD	
	Kernels planted	Mature plants	Kernels planted	Mature plants
56_2 tassel	7	3		
$56_2 \times 33_8$	10(3 died)†	4	21*	7
56_2 selfed			6	0
$21_{14} \times 56_2$			1	1
$23_8 \times 56_2$	2	2	6	4
$24_{18} \times 56_2$	2	2	2	2
$26_1 \times 56_2$	5(2 died)†	3	8	5
$30_{14} \times 56_2$			1	0
$33_8 \times 56_2$	1(died)†	0	2	2
$36_2 \times 56_2$	10(5 died)†	3	10	7
$38_8 \times 56_2$	5(1 died)†	4	3	2

* 9 well developed kernels gave rise to 4 mature individuals, one with $8^{II}+2^{III}$, one with $7^{II}+3^{III}$ and two with $6^{II}+4^{III}$.

12 underdeveloped kernels gave rise to 3 mature individuals with chromosome numbers $6^{II}+4^{III}$, $5^{II}+5^{III}$ and $3^{II}+7^{III}$ respectively.

† Plants began development but died from "damping off" in seedling stage.

MEIOSIS IN F_1 INDIVIDUALS

The behavior of the chromosomes during meiosis was studied in fifty F_1 individuals. When diploid individuals were pollinated with pollen from the triploid, twenty of the thirty-seven plants examined showed ten pairs of

chromosomes at diakinesis and metaphase *I*. The meiotic behavior in these plants was similar in all observed details to that in ordinary diploid individuals. In size and vigor they surpassed those F_1 individuals which possessed one or more extra chromosomes (compare plant No. 14, text figure 6 with its sib, plant No. 10, text figure 5; plants 15, 16 and 18, text figure 6 with their sibs, plants 11, 12 and 13, text figure 5).

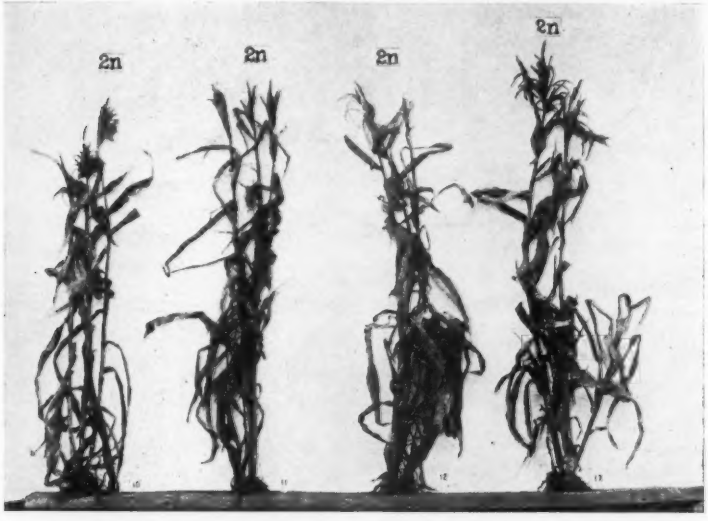

FIGURE 5.—Diploid individuals resulting from cross diploid ♀ × triploid ♂. Plant no. 10, 86_1; no. 11, 88_2; no. 12, 88_3; no. 13, 89_2.

Meiosis in 2n+1 individuals

Out of fifty F_1 individuals examined, eight showed the presence of one extra chromosome: two out of thirteen in the cross triploid ♀ × diploid ♂ and six out of thirty-seven in the reciprocal cross, diploid ♀ × triploid ♂. Cytological examination indicated that different chromosomes of the haploid set we involved in the several 2n+1 plants. However, the 2n+1 plants did not show characteristic differences that could be ascribed to certain chromosomes of the set.

In describing meiosis in these plants reference will be made, at times, to figures from plants which contained more than one extra chromosome. Since similar conditions existed in all the extra chromosome plants, one

figure will suffice for illustration. This has been done to avoid repetition of figures illustrating similar phenomena.

There were fewer meiotic irregularities in these individuals than in those containing more than one extra chromosome. As in all F_1 extra chromosome plants, a variation in synaptic expression of the extra chromosome was noticed. In the 2n+1 plants $9^{II}+1^{III}$ (figure 11, plate 2) was found about twice as frequently as $10^{II}+1^{I}$ (figures 12 and 13) in both diakinesis and metaphase *I* (table 4). The appearance and position of the univalent during metaphase *I* are rather varied. The univalent may be found in any region of the spindle. It occasionally is not included in the spindle but

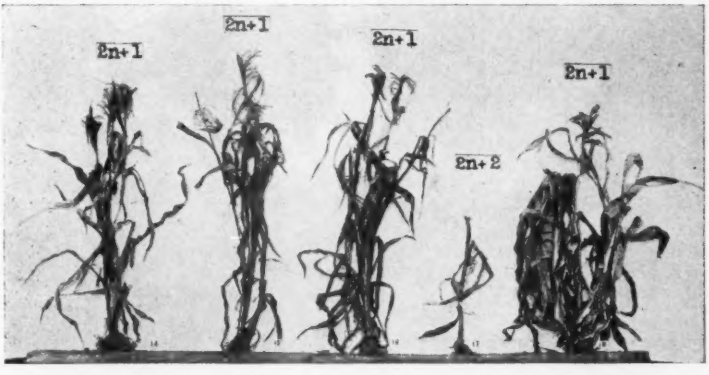

FIGURE 6.—Extra chromosome individuals resulting from cross diploid ♀ × triploid ♂. These are sibs of those plants shown in text figure 5. Plant no. 14, 86_2; no. 15, 88_1; no. 16, 88_3; no. 17, 88_4; no. 18, 89_1.

lies in the cytoplasm. When a univalent lies in or near the plate it may split simultaneously with the disjunction of the bivalents, or it may lag until the bivalents have completely disjoined and have passed toward either pole, when, in turn, it may split, the halves separating and advancing toward opposite poles. These may or may not be included in the reorganizing nuclei (figures 9, 10, 24, 28 and 34). The univalent may remain unsplit at the equator and subsequently be left in the cytoplasm as a chromatic mass. When a trivalent is present, on the other hand, disjunction of the three homologues and the passage of two toward one pole and one toward the other pole seem to be quite regular.

Interkinesis most frequently shows two well organized nuclei lying in cytoplasmic masses separated by a cell plate. Occasionally, in the cyto-

plasm of one or both cells, there lie one or more chromatic bodies distinguishable as dyad or monad. Besides the two major nuclei one or more very small nuclei may be found in the cytoplasm of one or both cells.

TABLE 4

Frequency of appearance of different synaptic combinations in F_1 extra chromosomes individuals.

PLANT	SYNAPSIS	DK.	M_I	PLANT	SYNAPSIS	DK.	M_I
86_2	$9^{II}+1^{III}$	8	28	94_3	$3^{II}+7^{III}$	5	8
	$10^{II}+1^{I}$	4	10		$4^{II}+6^{III}+1^{I}$	4	8
					$5^{II}+5^{III}+2^{I}$	2	5
88_1	$9^{II}+1^{III}$		5		$6^{II}+4^{III}+3^{I}$	2	2
	$10^{II}+1^{I}$	1	7		$7^{II}+3^{III}+4^{I}$		
					$8^{II}+2^{III}+5^{I}$		
88_3	$9^{II}+1^{III}$	9	40		$9^{II}+1^{III}+6^{I}$		2
	$10^{II}+1^{I}$	6	12		$10^{II}+7^{I}$		
89_1	$9^{II}+1^{III}$	1	7	95_1	$7^{II}+3^{III}$	1	33
	$10^{II}+1^{I}$		4		$8^{II}+2^{III}+1^{I}$	1	41
					$9^{II}+1^{III}+2^{I}$		27
	$8^{II}+2^{III}$	6	8		$10^{II}+3^{I}$		6
88_4	$9^{II}+1^{III}+1^{I}$	4	7				
	$10^{II}+2^{I}$	1	2	95_2	not recorded		
	$8^{II}+2^{III}$		20				
94_1	$9^{II}+1^{III}+1^{I}$	1	8				
	$10^{II}+2^{I}$		2				
					$6^{II}+4^{III}$		6
	$5^{II}+5^{III}$		7		$7^{II}+3^{III}+1^{I}$		11
	$6^{II}+4^{III}+1^{I}$		6		$8^{II}+2^{III}+2^{I}$		10
	$7^{II}+3^{III}+2^{I}$		3	95_3	$9^{II}+1^{III}+3^{I}$		3
	$8^{II}+2^{III}+3^{I}$	1	1		$10^{II}+4^{I}$		1
	$9^{II}+1^{III}+4^{I}$		2				
94_2	$10^{II}+5^{I}$		0		$6^{II}+4^{III}$		18
	$9^{II}+7^{I}$		1		$7^{II}+3^{III}+1^{I}$		12
	$4^{II}+3^{III}+8^{I}$	1	1	94_4	$8^{II}+2^{III}+2^{I}$		4
	$3^{II}+2^{III}+13^{I}$	1			$9^{II}+1^{III}+3^{I}$		
	$2^{II}+1^{III}+18^{I}$		1		$10^{II}+4^{I}$		
	$1^{II}+1^{III}+20^{I}$		1				
	25^{I}		3				

Dk. = diakinesis; MI = metaphase I.

Since there were many 10–11 anaphase I chromosome distributions, this same relation appeared in pairs of prophase II nuclei. The second meiotic mitosis proceeded with little irregularity. Occasionally a dyad chromosome lagged in anaphase II with or without subsequent separation of its members; or, as the anaphase advanced, a monad ceased to keep pace and was

left out of the telophase nucleus. Sometimes such a lagging chromosome, whether a dyad or a monad, became included in the forming wall (figure 30). Some cells possessed a second small spindle, oriented at various angles or connected with the major spindle.

In consequence of such meiotic behavior we most frequently encountered complete quartets of spores, each spore containing one well formed nucleus. Occasionally other small nuclei or chromatic bodies were observed in the cytoplasm. Only seldom did a sporocyte give rise to more than four spores.

Unusual meiotic phenomena in 2n+1 individuals

Some anther loculi in both greenhouse and field plants were filled with a plasmodial mass including either prophase *I* nuclei, like or unlike in size, or metaphase *I* figures, free or connecting with each other at various angles (figure 19). The chromosome number in the various figures was not always the same. Some had more than the normal number for the plant in question and some less (upper spindle, figure 19). In one case the number amounted to four times that expected, the spindle being perfectly formed and the chromosomes regularly arranged. There appeared, in a few of these plasmodial sporogenous masses, nuclei containing amounts of chromatin not in accordance with their various sizes. A large nucleus might contain but a single chromatic thread lying against the nuclear membrane, nearly the whole volume, of the nucleus being karyolymph. An adjacent nucleus, small in volume might be densely packed with a chromatin spireme. In the same plasmodium all states between these two existed.

In figure 17 is shown a metaphase *I* spindle containing $20^{II}+2^{I}$, double the expected number; this was seen in a well rounded sporocyte. Figure 18 shows a well formed spindle containing $40^{II}+4^{I}$, or four times the expected number.

Fragmentation appeared in these and other F_1 plants. It was most evident during anaphase *I* (figure 20). The fragments vary in size from a mere speck of chromatin to a large portion of a dyad. Appearances indicate that some of these fragments have the ability to divide. This is observable in the chromosome fragments at the center of the spindle (figure 20).

Meiosis in 2n+2 individuals

Out of fifty F_1 individuals examined, six possessed two extra chromosomes belonging to two different homologous groups. In the direct cross triploid ♀ × diploid ♂ four out of thirteen showed two extra chromosomes; in the reciprocal cross, two out of thirty-seven. These plants were smaller and less vigorous than their sibs with one extra chromosome.

TABLE 5 (Abridgment of Table 1)

CROSS	NUMBER OF KERNELS ON EAR	NUMBER OF KERNELS PLANTED	NUMBER OF PLANTS DEVELOPED	2n 10^{II}	2n+1 $9^{II}+1^{III}$	2n+2 $8^{I}+2^{III}$	2n+3 $7^{II}+3^{III}$	2n+4 $6^{?I}+4^{III}$	2n+5 $5^{II}+5^{III}$	2n+6	2n+7 $3^{II}+7^{III}$	2n+8	2n+9	3n 10^{III}	2n+2† 11^{II}
56_2 tassel	22	7	3		1	1	1								
$56_2 \times 33_8$	34	31	11*		1	3	1	3	1		1				
56_2 selfed	6	6	0												
Total		44	14		2	4	2	3	1		1				
$21_{14} \times 56_2$	1	1	1	1											
$23_8 \times 56_2$	9	8	6	6											
$26_1 \times 56_2$	22	13	8	6	1									1	
$24_{18} \times 56_2$	6	4	4	3											1
$30_{14} \times 56_2$	1	1	0												
$33_5 \times 56_2$	3	3	2	2											
$36_2 \times 56_2$	49	20	10	3	5	2									
$38_8 \times 56_2$	18	8	6	6											
Total		58	37	27	6	2								1	1
Grand Total		102	51	27	8	6	2	3	1		1			1	1

* Chromosome number of one plant not obtained.
† See note in table 2.

Meiosis in these plants was similar in most details to that of the 2n+1 individuals with the added complication of the one extra chromosome; thus making the observed irregularities more numerous than in the 2n+1 plants. The univalents acted similarly but independently of one another. During diakinesis and metaphase *I* (figure 22, plate 3) were seen most frequently $8^{II}+2^{III}$, less frequently $9^{II}+1^{III}+1^{I}$ (figure 21) and least frequently $10^{II}+2^{I}$ (see table 4).

Because of the extra chromosome, anaphases were markedly more irregular than in the 2n+1 plants. Here we frequently found 11 chromosomes passing to each pole, although 10–12 distributions were numerous. The lagging of one or two chromosomes, the lagging and splitting of one or two, or combinations of these two types were rather numerous. Figure 24 illustrates the lagging and splitting of one dyad in a spindle where ten chromosomes have gone to one pole and eleven to the opposite pole. Appearances in telophase *I* and prophase *II* were similar to those in the 2n +1 plants. Figure 31, a prophase *II*, in which the chromosomes in the left cell are farther advanced than those in the right, indicates that a 10–12 distribution in anaphase *I* must have taken place. Such a prophase as that in the right half of figure 31 might be followed by an anaphase *II* distribution like that illustrated in figure 29. More frequently, however, the anaphases of *II* were less regular, being marked by the lagging of one or two chromosomes which eventually become excluded from the reorganizing nuclei. In consequence, the spores frequently appeared much as in figure 30. Sometimes an irregular spore quartet, as illustrated in figure 25, was formed.

One greenhouse plant (68_3) exhibited more striking irregularities in meiotic phenomena than any other individual examined. Many anthers possessed one continuous plasmodium in which lay nuclei ranging in size from very minute to those two and three times the volume of the normal nucleus. These, in turn, probably gave rise to plasmodial sporogenous masses in which lay spindles of all sizes containing from very few chromosomes to more than four times the normal number for the plant. In one huge sporocyte there were two parallel metaphase *I* spindles, in each of which lay four times the normal number of chromosomes. The chromosomes were associated as bivalents, trivalents, quadrivalents, quintivalents and sexivalents. Unfortunately, the slide was damaged before a drawing was made.

In this plant some sporocytes exhibited metaphase *I* figures with twice the normal number of chromosomes. In all previous cases in other plants where tetraploid metaphase *I* spindles were observed, the chromosomes

of the four sets gave no synaptic evidence of homology (see figures 17 and 18). Here, however, the homologies were frequently expressed by the synaptic union of four chromosomes to form a quadrivalent or of six to form a sexivalent (it is to be remembered that this plant showed, normally, $8^{II}+2^{III}$). Not all the chromosomes of the four sets necessarily formed such synaptic combinations, for two homologous bivalents sometimes appeared distinctly separated. Such a case is illustrated in figure 27, which was drawn from a remarkably clear figure with $2^{VI}+6^{IV}+4^{II}$. Figure 28 represents an anaphase *I* distribution of chromosomes in a tetraploid sporocyte including a lagging bivalent, a lagging and splitting dyad and two lagging but separating monads.

Figure 26 shows a metaphase *I* spindle from this same plant which contains 1 bivalent less than normal, that is, $7^{II}+2^{III}$.

Meiosis in plants with 2n+3 to 2n+7 chromosomes

A reference to table 5 will show that individuals with more than two extra chromosomes appeared only in the cross triploid ♀ × diploid ♂ (with the exception of a new triploid). There were two plants with $7^{II}+3^{III}$, three with $6^{II}+4^{III}$, one with $5^{II}+5^{III}$, and one with $3^{II}+7^{III}$. The presence of extra chromosomes was suggested in the small size and decreased vigor of these F_1 individuals before meiosis was observed (see text figure 3).

Since synapsis was so varied, it was of interest to determine the percentage of bivalents, trivalents and univalents in the various diakinesis and metaphase *I* figures of each plant. Reference to table 4 will show that there was a general tendency on the part of the univalents to be associated with the bivalents to form trivalents at diakinesis and metaphase *I* in a good percentage of the cases.

Figure 32, plate 4, shows $7^{II}+3^{III}$, while figure 33 represents a metaphase *I* with $8^{II}+2^{III}+1^{I}$. In figure 36 we observe $8^{II}+1^{III}+4^{I}$, indicating that there has been non-synapsis or early disjunction of one of the bivalents ordinarily present as such. A metaphase *I* figure in plant 95_2 with four extra chromosomes is shown in figure 39; here there are clearly $6^{II}+4^{III}$.

Plant 95_3 with four extra chromosomes is represented by figure 37, in which we find $6^{II}+4^{III}$. When it was determined that photography could be of service in describing the results, this same figure was photographed (figure 14, plate 6). In an adjacent sporocyte (text figure 7) we find $7^{II}+3^{III}+1^{I}$, while in still another sporocyte we see $10^{II}+4^{I}$ (figure 15, plate 6).

A metaphase *I* with $3^{II}+7^{III}$ is shown in figure 38, which was drawn from a sporocyte of a 2n+7 individual.

Anaphases in these plants were very similar to those already described

for other F_1 extra-chromosome individuals, with the added complication of more univalents and consequently more irregular behavior. In an anaphase *I* (figure 34) of plant 95_1 (2n+3) two lagging dyads show clearly the split in preparation for *II*, while the components of two other dyads have already commenced to separate.

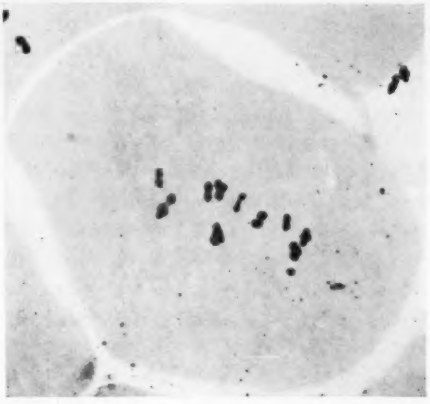

FIGURE 7.—Photomicrograph of microsporocyte of 2n+4 individual showing 7 bivalents, 3 trivalents and 1 univalent. See photographs of adjacent sporocytes, figures 14 and 15, plate 6. From an aceto-carmine smear preparation.

Since irregularity increases in proportion to the number of extra chromosomes present, the variation in spore formation increases accordingly. Giant spores, dyads, quartets and polyspory with and without extra, small nuclei or chromatin masses were repeatedly observed.

F_1 triploid plant

In the greenhouse plants there appeared a new triploid from the cross diploid ♀ × triploid ♂. Since meiosis in the new triploid was essentially similar to that of the triploid already described, no further discussion will be given. Figure 40 represents a metaphase *I* in which there are 10^{III}.

Irregular synapsis in a 2n+5 individual

Since plant 94_2 (2n+5) showed an almost complete series of nonsynapses of homologous chromosomes during diakinesis and metaphase *I*, ranging from $5^{II}+5^{III}$ to 25^{I}, it has been considered separately. In many of the sporocytes of an anther we found the synaptic condition to be very similar to that already described for other extra-chromosome F_1 indivi-

duals, that is, $5^{II}+5^{III}$, $6^{II}+4^{III}+1^{I}$ to $10^{II}+5^{I}$ (figure 41). Besides this, non-synapsis extended to the bivalents themselves, forming, for instance, $9^{II}+7^{I}$ (figure 42) or $4^{II}+3^{III}+8^{I}$ (figure 43).

The appearance of the diakinesis figures (figure 43) points toward a possible early disjunction as an explanation, since the members of many bivalents and trivalents were specially far apart, remaining connected only by long, fine threads.

Anaphases vary in appearance according to the number of univalents present. When there are few univalents the figures are quite normal. When there are many univalents there is no well organized equatorial plate (figure 45, $1^{II}+23^{I}$; figure 46, $1^{II}+1^{III}+20^{I}$; figure 44, 25^{I}) the chromosomes being distributed throughout a greater part of the spindle.

We have, at present, no satisfactory explanation of this appearance. It is difficult to explain why such wide variations in synaptic expression occur in sporocytes of the same anther or of other anthers at the same stage of development. In the summer of 1926, under field conditions, RANDOLPH (1928) examined a number of inbred strains which were showing this non-synaptic phenomenon in many sporocytes. Normally in these individuals there are 10^{II} at metaphase *I* and meiosis is regular. However, in those diploid plants which showed some sporocytes with varying degrees of non-synapsis the univalents thus formed behaved much as they did in the extra-chromosome F_1 individuals and in hybrids in general. They lagged, or lagged and split, giving appearances of irregularity in a number of sporocytes. Other sporocytes in the same anther were perfectly normal in appearance and behavior.

Non-synapsis at diakinesis, whether it represents a true non-synapsis or merely an early disjunction, is caused in some cases by conditions other than lack of homology of the chromosomes present.

The F_1 extra-chromosome individuals described in this paper have been selfed and crossed for further cytological investigations to be reported later.

GENETIC INVESTIGATIONS

The kernels of culture 56 in which the triploid plant arose were planted for use as a sugary tunicate stock. In consequence, no particular attention was paid to the color of the kernels other than to note that a segregation for purple and white aleurone had occurred on the ear from which they had come. Since the culture was also segregating for the plant color factor *A*, little could be concluded directly about the constitution of the triploid itself. It was known to be phenotypically dilute sun red, tunicate and sugary.

Since three sets of chromosomes were present, it was desired to know what the genetic constitution of the triploid might be with respect to certain genetic factors, and how these might be carried to future generations. For this reason pollen of the triploid was placed upon silks of several genetic "testers" (see table 1) whose mirosporocytes had been examined to detect the possible presence of extra chromosomes or irregular behavior.

One ear of the triploid plant was used for the cross $56_2 \times 33_5$, the reciprocal cross ($33_5 \times 56_2$) being made also. A second ear was selfed and a third ear on a tiller was used in the cross triploid × tetraploid (perennial teosinte).

It will be seen by an examination of table 1 that very few kernels developed as a result of most of the crosses. If we disregard the possible selective germination of the F_1 kernels carrying extra chromosomes, it will appear on examination of table 5 that in most crosses the male gametes from the triploid which functioned possessed ten chromosomes.

In the crosses $21_{14} \times 56_2$, $23_8 \times 56_2$ and $33_5 \times 56_2$ (table 5) very few kernels developed on the ears. All the plants that grew to maturity from these crosses possessed but ten pairs of chromosomes. In certain crosses, namely, $26_1 \times 56_2$ and $36_2 \times 56_2$ (table 5) where relatively more kernels developed on an ear, the F_1 plants coming from these kernels showed not only diploids but some 2n+1 and 2n+2 individuals. This suggests that in the former case only the pollen grains carrying the haploid set of chromosomes functioned, while in the latter case not only the haploid pollen functioned but also the haploid+1 and some of the haploid+2 pollen, therefore producing more kernels on the ears.

Before beginning a discussion of the results obtained it would be well to outline briefly the expected diploid × triploid ratios, assuming random assortment of the extra chromosomes in the triploid with no selection of pollen or of F_1 chromosome combination. It is known from the observations of meiosis in the triploid that pollen carrying various chromosome numbers was formed. Assuming, for descriptive purposes, a factor *A* and its allelomorph *a*, we can say that if the triploid were homozygous for *A* (*AAA*), that is, triplex (BLAKESLEE, BELLING and FARNHAM 1920), the F_1 would be, phenotypically, *A*. If the triploid had the constitution *AAa* (duplex) the expected gametic ratio would be 2*A*: 1*AA*: 2*Aa*: 1*a*, and when crossed to *a* it would give, phenotypically, 5*A*: 1*a*. However, if the triploid were *Aaa* (simplex) the gametic ratio would be 1*A*: 2*Aa*: 2*a*: 1*aa*, and the backcross phenotype ratio 1*A*: 1*a*. If the triploid were homozygous recessive (nulliplex) the backcross would be totally recessive, phenotypically.

If, now, there is a decided selection against the gametes carrying extra chromosomes, the functional gametic ratio becomes modified accordingly. In the case of *AAa* (duplex) elimination of the extra chromosome carrying gametes would bring the functioning gametic ratio to 2*A*: 1*a*. In the case of *Aaa* (simplex) such a selection would give a functioning gametic ratio of 1*A*: 2*a*. Gametic selection in the reciprocal crosses of the diploid × triploid has been discussed in connection with the cytology of the F_1 individuals. We have seen that meiosis in diploid individuals usually results in the production of haploid spores and in consequence haploid gametes containing ten chromosomes. Meiosis in the triploid, however, results in the production of microspores containing various chromosome numbers. When pollen from the triploid is used in crossing on to silks of a diploid individual the pollen grains that seem to be functionally successful contain for the most part 10 chromosomes, or occasionally 10+1 or 10+2. Hence, from such a cross we should expect a duplex or simplex factorial condition in the triploid to appear in the backcross in the phenotypic ratios of 2*A*: 1*a* or 1*A*: 2*a* respectively. In the cross triploid ♀ × diploid ♂, on the other hand, we have seen that there has been no such marked elimination of the extra chromosome carrying gametes of the triploid. In consequence the ratios should be expected to show some approach to those indicated in the preceding paragraph.

Although not all the F_1 kernels have been grown and the genetic data are few, the results so far obtained and outlined below tend to confirm this expectation.

The triploid was triple recessive or nulliplex for the plant color factors *b* and p_l (Emerson 1921); since it was dilute sun red and gave only dilute sun red individuals when crossed to plants which were homozygous *Abp*$_l$. Likewise it was nulliplex for the factor s_u responsible for sugary endosperm (see table 1, $36_2 \times 56_2$ and 56_2 selfed) and the endosperm factor *y* which is the allelomorph of *Y* responsible for yellow endosperm (see table1, $36_2 \times 56_2$) (Correns 1901, East and Hayes 1911).

The evidence points toward the fact that the triploid was simplex for the factors *C* and *R*, two complementary aleurone color factors (Emerson 1918). Considering the factor *C*, which with *A* and *R* seems to be responsible for the presence of colored aleurone, the following ratio of F_1 kernel color was noted when the triploid was crossed to an *AAccRR* or so-called *C*-tester (table 6).

Since in these crosses there was a selection against pollen carrying the higher numbers of chromosomes expected on the assumption of random assortment, the F_1 ratio, according to our interpretation, should approach

TABLE 6

CROSS *AAccRR*×triploid	F_1 ALEURONE COLOR	
	Colored	Colorless
$24_{18}\times56_2$	4	1
$36_2\times56_8$	19	30
Total	23	31

a 1:2 if the triploid was simplex for the factor *C*. Although the data are few, this ratio is approximated. Certainly it does not approach the duplex 2:1 ratio.

With reference to the factor *R*, the simplex condition in the triploid was suggested by the results of the following crosses which were made to *R* testers (*AACCrr*) (table 7).

TABLE 7

CROSS *AACCrr*×triploid	F_1 ALEURONE COLOR	
	Colored	Colorelas
$17_2\times56_2$	9	8
$25_1\times56_2$		2
$26_1\times56_2$	9	13
$38_8\times56_2$	4	14
Total	22	37

Since the functioning male gametes are predominantly carrying only ten chromosomes (table 5), the results recorded in table 7 can best be interpreted as a 1:2 modified simplex ratio.

Fortunately, the triploid possessed the dominant plant factor tunicate (T_u) COLLINS 1917). Direct and reciprocal crosses were made to the double recessive (t_ut_u). Since there was no obvious selection against gametes carrying higher chromosome numbers in the cross triploid ♀×diploid♂, the unmodified triploid ratios would be expected to appear in the F_1 of this cross, that is, if the triploid were simplex for T_u, the ratio would be a 1:1, if duplex, 5:1. In the reciprocal cross, since selection against the higher chromosome gametes occurs, the modified ratio should appear, that is, if simplex a 1:2, if duplex, a 2:1 ratio. The following are the results obtained in the direct and reciprocal crosses (tables 8 and 9).

TABLE 8

F_1 triploid ♀ × *diploid* ($t_u t_u$) ♂.

CROSS	TUNICATE	NON-TUNICATE
$56_2 \times 33_8$	8	2

TABLE 9

F_1 diploid ($t_u t_u$) ♀ × *triploid* ♂.

CROSS	TUNICATE	NON-TUNICATE
$21_{14} \times 56_2$	0	1
$23_3 \times 56_2$	2	3
$24_{18} \times 56_2$	2	2
$26_1 \times 56_2$	7	1
$33_5 \times 56_2$	1	1
$36_2 \times 56_2$	7	3
$38_3 \times 56_2$	4	2
Totals	23	13

The results tabulated above afford evidence for a duplex condition of T_u ($T_u T_u t_u$) in the triploid. Although the number of plants is small, the data in the two crosses in no way conflict. On the contrary, the two crosses act as decided checks upon one another and upon the interpretation assumed to explain modified ratios. Where no modified ratio is expected, that is, where no decided gametic selection has occurred, the duplex 5:1 ratio is approached (table 8). In the reciprocal cross, moreover, a duplex modified ratio of 2:1 is approximated where gametic selection is known to have occurred (table 9).

The plant anthocyan color factor *A* was present in the triploid as either simplex or duplex. Unfortunately one cross ($16_9 \times 56_2$, table 1) made to test this factor produced no kernels. The second cross ($23_8 \times 56_2$, table 1) produced but 9 kernels, 4 colored: 5 colorless. The results merely indicate that the triploid plant was heterozygous for *A*, the numbers being too low to decide between the simplex and the duplex condition.

From what has been stated concerning the inheritance of the factors mentioned above, we are able to reconstruct, to some extent, the constitution of the triploid with regard to these factors as follows: *A?a bbb* $p_1 p_1 p_1$ $T_u T_u t_u$ $s_u s_u s_u$ *Ccc Rrr*.

DISCUSSION

Among investigations on plants several cases of trisomic inheritance have been observed. At present "trisomic inheritance" has two meanings in the literature, both of which, however, depend upon the presence of three instead of two homologous chromosomes.

In the first category the character followed in inheritance depends simply upon the presence of the extra chromosome for its expression. The 2n+1 chromosomal complement produces a particular recognizable character in a plant as contrasted with the 2n condition, and this character may be followed without special reference to the genic constitution of the extra chromosome as compared with that of its homologues. If a 2n+1 individual is crossed on to a 2n individual the phenotypic ratio in F_1 depends solely upon the relative number of individuals which do or do not possess this extra chromosome. Thus, in this type of trisomic inheritance it is the distribution of the extra chromosome that is followed through successive generations.

This kind of inheritance, depending upon an extra chromosome for the expression of a mutant character, has been extensively investigated (BLAKESLEE et al.) in the Datura mutant types Globe (2n+1), Poinsettia (2n+1) etc., each of these types being the result of a duplication of a different chromosome of the set. Other examples are the Nicotiana mutant "enlarged" flower (2n+1) (CLAUSEN and GOODSPEED 1924), the Matthiola mutants Large, Slender, Smooth, Crenate, etc, (FROST and MANN 1924; FROST, 1927), and 2n+1 types in Lycopersicum (LESLEY 1928).

In Zea any one particular chromosome when it is present in triplicate in 2n+1 individuals has not produced, so far as we have noticed, any one particular recognizable character. The presence of extra chromosomes was recognizable, phenotypically, only in decreased size and vigor.

In the second category would come the inheritance of characters which depend upon a factor located in an extra chromosome in a triploid or a 2n+1 individual. It is essentially Mendelian. The ratios obtained in inheritance of the first category are expressions merely of the relative number of 2n and 2n+1 individuals. Those in the second category follow the accepted laws of Mendelian inheritance on a trisomic rather than a disomic basis. In most cases the formulation of an expected ratio would depend upon the assumption of a random assortment at meiosis of the three homologous chromosomes containing the factors in question, and a perfectly random mating. In all cases such expected ratios must be modified by any selective action against certain extra-chromosomal gametic types.

This genic type of trisomic inheritance has been studied in the case of purple and white flower factors in the Poinsettia chromosome of Datura. Recently other gene characters (B-white, swollen, tricarpel) on other chromosomes have been studied (BLAKESLEE 1927; BUCHHOLZ and BLAKESLEE 1927; GAGER and BLAKESLEE 1927). In these cases both types of trisomic inheritance are exhibited together. Trisomic inheritance in Zea, as reported in the present study, is of the second type as indicated by the inheritance of the factors T_u, C and R. LESLEY (1926) has found similar trisomic gene inheritance in tomato.

Among animals the inheritance in chromosomal types other than the diploid has been rather extensively investigated in Drosophila (BRIDGES 1916, 1921, 1922; L. V. MORGAN 1922, 1925; ANDERSON 1925; BRIDGES and ANDERSON 1925).

With regard to Zea it should be kept in mind that the data are necessarily few owing (1) to the limited amount of pollen produced bythe triploid and thus the limited number of crosses that could be made, (2) to the great sterility of this pollen and consequently the few kernels that resulted from each cross, and (3) to the inability to recognize chromosomal types other than by a cytological examination. Hence, the amount of material grown was limited strictly to that which could be studied by one observer in a certain period of time.

SUMMARY

1. A triploid *Zea mays* individual was found in a culture which was otherwise diploid.

2. Meiosis in the microsporocytes of this individual is described. The basic chromosome number in Zea is believed to be ten. At diakinesis and metaphase of the first meiotic mitosis the triploid frequently showed ten trivalents. There was a tendency for members of the extra set of chromosomes to be disassociated from their homologues at diakinesis and metaphase, thus forming nine trivalents, one bivalent and one univalent or eight trivalents, two bivalents and two univalents etc.

3. Reciprocal crosses were made with diploid Zea.

4. Observations on fifty F_1 individuals of the reciprocal crosses of diploid $\times$ triploid were made. It was found that there had been a decided selection against extra chromosome carrying male gametes and a less obvious selection against extra chromosome carrying eggs.

5. The presence of extra chromosomes in Zea plants, other than in a polyploid series, is associated with a decrease in size and vigor.

6. Ratios for several factors (T_u, R and C) were found to accord with the theoretical expectancy on the basis of trisomic inheritance.

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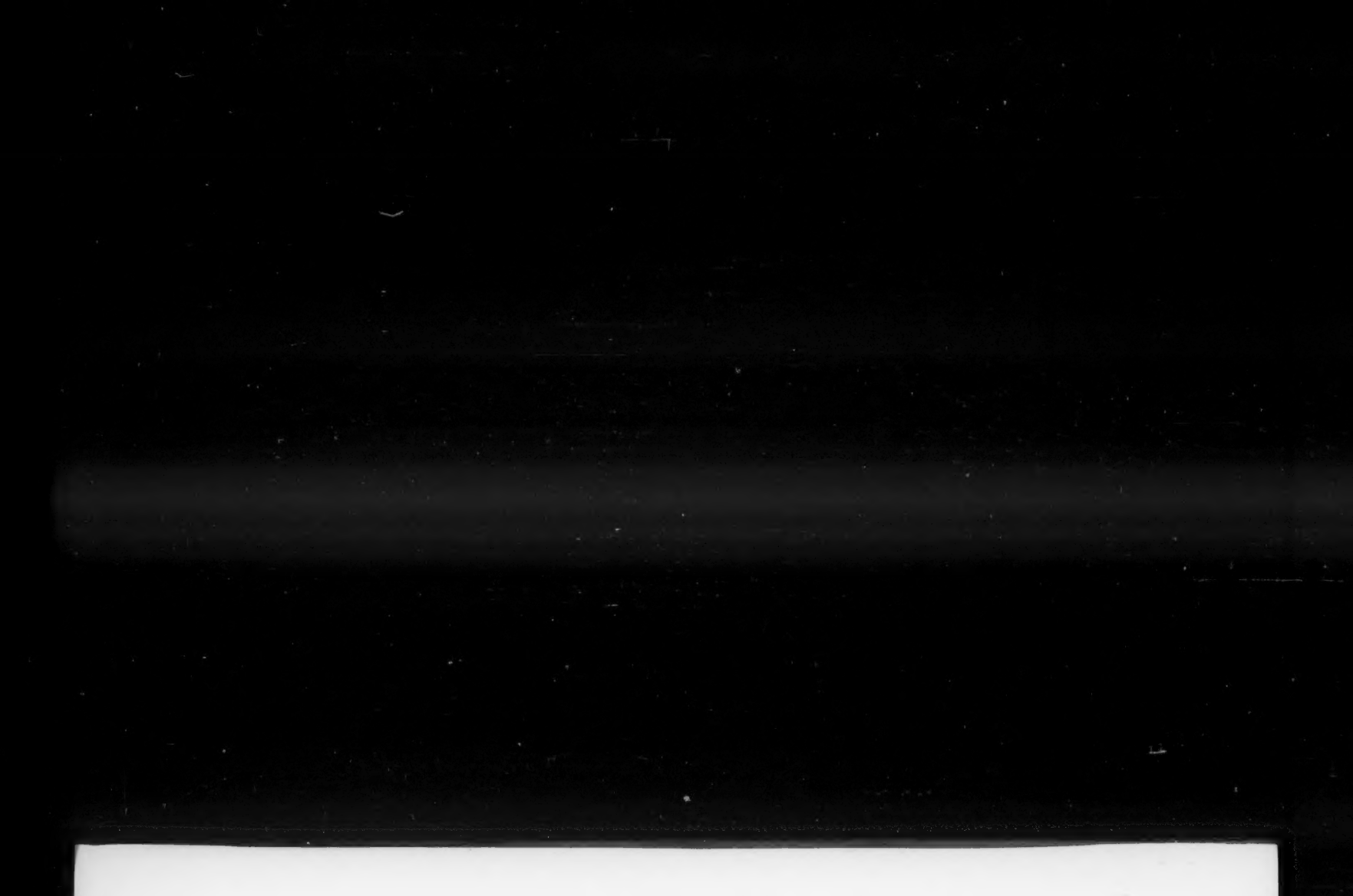
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